EXHIBIT 1

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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

TAKEDA PHARMACEUTICAL COMPANY LIMITED, and TAKEDA PHARMACEUTICALS U.S.A., INC. Plaintiffs, v. NORWICH PHARMACEUTICALS, INC. Defendant.	)	C.A. No. 20-cv-8966-SRC-CLW

MARK D. HOLLINGSWORTH, PH.D.'S DECLARATION IN SUPPORT OF DEFENDANT'S OPENING CLAIM CONSTRUCTION BRIEF

I, Mark D. Hollingsworth, Ph.D., hereby declare and state as follows:

I. INTRODUCTION

1. I have been retained by Norwich Pharmaceuticals, Inc. ("Norwich"), in the above matter. I understand that Takeda Pharmaceutical Company Limited and Takeda Pharmaceuticals U.S.A., Inc. ("Takeda") have asserted against Norwich U.S. Patent Nos. 7,105,486 ("the '486 patent"), 7,223,735 ("the '735 patent"), 7,655,630 ("the '630 patent"), 7,659,253 ("the '253 patent"), 7,659,254 ("the '254 patent"), 7,662,787 ("the '787 patent"), 7,662,788 ("the '788 patent"), 7,671,030 ("the '030 patent"), 7,671,031 ("the '031 patent"), 7,674,774 ("the '774 patent"), 7,678,770 ("the '770 patent"), 7,678,771 ("the '771 patent"), 7,687,466 ("the '466 patent"), 7,687,467 ("the '467 patent"), 7,700,561 ("the '561 patent"), 7,713,936 ("the '936 patent"), 7,718,619 ("the '619 patent"), and 7,723,305 ("the '305 patent") (collectively, "the Patents-in-Suit").

2. Counsel for Norwich has asked me to provide my opinions on the meaning of the terms (1) "L-lysine-d-amphetamine mesylate," "mesylate salt of L-lysine-d-amphetamine," and ". . . wherein said salt is a mesylate salt" (collectively, "the 'mesylate' terms") as recited in the '486 patent (claim 4), the '735 patent (claim 3), the '254 patent (claim 3), the '787 patent (claim 3), the '788 patent (claims 5, 22, 27, 34, 41, 46), the '030 patent (claims 3, 10, 17, 24, 31, 38), the '031 patent (claims 6, 22), the '774 patent (claims 3, 10, 17, 24, 31, 38),

the '770 patent (claim 4), the '771 patent (claims 3, 10, 17), the '466 patent (claim 10), the '467 patent (claims 3, 10, 17, 24, 31, 38), the '936 patent (claims 6, 22, 38, 54), the '619 patent (claims 3, 10, 17, 24, 31, 38), and the '305 patent (claims 3, 10, 17, 24, 31, 38) and (2) "isolated" in the '787 patent (claims 1, 2).

3. I am being compensated for my time at the rate of $500 per hour. Additionally, I am being reimbursed for my expenses incurred in preparing this report. My compensation is in no way contingent on the outcome of the case or on any of my positions or opinions concerning any issue in the case.

4. In forming my opinions, I have reviewed the materials listed in Exhibit A. I reserve the right to amend or supplement this list with any inadvertently omitted materials.

II. SUMMARY OF OPINIONS

5. In my opinion, the "mesylate" terms, when read in light of the specification and prosecution history, mean "a salt with any number of mesylate ions associated with it."

6. In my opinion, the term "isolated," when read in light of the specification and prosecution history, means "non-salt form."

III. QUALIFICATIONS

7. My curriculum vitae describes my educational background and experience, my technical expertise, and my publications. A copy of my curriculum vitae is attached as Exhibit B.

8. I earned my Ph.D. in organic chemistry from Yale University in 1986. My dissertation, which focused on reactions in organic crystals, was awarded several honors, including the 1986 Wolfgang Memorial Prize (best chemistry dissertation at Yale), the 1987 Nobel Laureate Signature Award for Graduate Education in Chemistry from the American Chemical Society (best chemistry dissertation in the U.S.), and the Distinguished Dissertation Award from the Northeastern Association of Graduate Schools (most distinguished dissertation in physical sciences and engineering from 1983-1987 in a consortium of approximately 60 graduate schools in the northeastern United States).

9. From November 1985 until August 1987, I conducted postdoctoral research at the University of Cambridge. My work focused on solid-state photochemistry, emphasizing the structural and dynamic characterization of reactive intermediates generated from organic molecules trapped in zeolites and organic inclusion compounds.

10. After completing my postdoctoral work, I began my independent academic career in 1987 as an Assistant Professor in the Chemistry Department at

the University of Alberta in Edmonton, Alberta, Canada. From September 1991 until August 1998, I was an Assistant Professor in the Chemistry Department at Indiana University in Bloomington, Indiana. Between 1991 and 1993, I was a Fellow of the Alfred P. Sloan Foundation. Currently, I am an Associate Professor in the Chemistry Department of Kansas State University in Manhattan, Kansas, a position I have held since 1998. In addition, I have been a Visiting Professor in the Department of Physics at University of Rennes in France, twelve times between 2001 and 2018, and I was a Visiting Professor in the Department of Chemistry at the University of Bordeaux, also in France, in 2006.

11. My research has focused on the following areas: (a) processes to obtain organic compounds in the solid state; (b) analytical techniques for characterizing organic solids and the processes that occur within them; (c) solid-state chemistry (broadly defined as the study of various solid forms of chemical compounds); and (d) mechanistic studies of crystal growth and polymorphism. Throughout my career, my group members and I have used organic synthesis to prepare the compounds that we study in the solid state, including methods such as crystallization, filtration, washing, and drying.

12. During the course of my work, I have used a variety of techniques to analyze the forms of crystalline materials. These include, but are not limited to, infrared and Raman spectroscopy, melting point, X-ray diffraction (single crystal

and powder), differential scanning calorimetry, thermogravimetric analysis, optical microscopy (including birefringence mapping), and solid-state and solution phase nuclear magnetic resonance.

13. During my academic career, I have taught more than 20 different undergraduate and graduate courses. As a professor of organic chemistry, I have taught the theory and practice of crystallization of organic compounds in numerous undergraduate laboratory courses. In addition, I have taught the section that deals with the mechanistic aspects of crystal growth of organic compounds in our graduate course in Materials Chemistry. I have supervised the research of 25 Masters, Ph.D., and postdoctoral students. Much of this research includes the study and isolation of particular crystal forms (polymorphs).

14. I have published extensively in the field of crystalline organic materials in peer-reviewed journals, including articles and book chapters as well as meeting presentations dealing with the isolation of new crystal forms, development of processes to obtain these forms, characterization of these solid forms, and chemical and physical processes that occur in crystalline materials. This list of publications includes seven in *Science* and *Nature*.

15. I have been a peer reviewer for a wide variety of journals, including *Science*, *Nature*, *Nature Chemistry*, the *Journal of the American Chemical Society*, *Angewandte Chemie*, *Advanced Functional Materials*, the *Journal of*

Pharmaceutical Sciences, the *Journal of Organic Chemistry*, *Crystal Growth and Design*, *CrystEngComm*, *Chemistry of Materials*, and *Molecular Pharmaceutics*. I have been a guest co-editor for special issues of *Molecular Crystals and Liquid Crystals*, *Chemistry of Materials*, and *Crystal Growth and Design*.

16. During the previous four years, I have testified as an expert at trial or by deposition in the following cases:

- *Bayer Healthcare LLC, Bayer Healthcare Pharmaceuticals Inc., and ONYX Pharmaceuticals, Inc. v. Mylan Pharmaceuticals Inc. and Mylan Inc.*; U.S. District Court Delaware, Case No. 15-114-LPS;
- *AstraZeneca, LP, AstraZeneca AB, AstraZeneca UK Limited, and AstraZeneca Pharmaceuticals LP v. Sigmapharm Laboratories, LLC, et al.*, U.S. District Court Delaware, Case No. 15-1000-RGA (Consolidated);
- *Boehringer Ingelheim Pharmaceuticals, Inc. and Boehringer Ingelheim International GMBH v. Sun Pharmaceutical Industries, Ltd. And Sun Pharmaceutical Industries, Inc.*, U.S. District Court New Jersey, Case No. 17-8819-MAS-LHG;
- *Forest Laboratories, LLC (f/k/a Forest Laboratories, Inc.), Forest Laboratories Holdings Limited, Allergan USA, Inc., and Pierre Fabre Medicament S.A.S. v. Aurobindo Pharma USA, Inc. and Aurobindo Pharma Limited, MSN Laboratories Private Limited and MSN Pharmaceuticals Inc.,*

Torrent Pharmaceuticals Limited and Torrent Pharma Inc., Hikma Pharmaceuticals USA Inc. and Hikma Pharmaceuticals International Limited, and Zydus Pharmaceuticals (USA) Inc., U.S. District Court New Jersey, Case No. 2:17-10230-ES-SCM (Consolidated);

- *H. Lundbeck A/S, Takeda Pharmaceutical Co. Ltd, et al. v. Apotex Inc. et al.*, U.S. District Court Delaware, Case No. 18-088-LPS (Consolidated); and
- *Re: Celgene Corp. v. Breckenridge Pharmaceutical, Inc., et al.*, U.S. District Court New Jersey, Case No. 19-5804.

IV. LEGAL STANDARDS

17. I am not a lawyer. As such, counsel for Norwich has provided me with a basic overview of the legal concepts relevant to claim construction.

A. Claim Construction

18. I understand that for claim construction purposes, the Court will look to the meaning a person having ordinary skill in the art ("POSA") at the time of the invention would have ascribed to the disputed claim terms.

19. I understand that to determine how the skilled person would have understood disputed claim language, courts first look to the "intrinsic evidence," the words of the claims themselves, the patent specification, and the prosecution history of the patent and its family members.

20. I understand that courts may also consider "extrinsic evidence," including all evidence external to the patent and prosecution history, such as dictionaries and learned treatises, and expert testimony concerning relevant scientific principles, the meanings of technical terms, and the state of the art. I understand that while courts may rely on extrinsic evidence, it is less significant than the patent and prosecution history in determining the meaning of claim language. I understand that extrinsic evidence can also be helpful to assist the Court in understanding what a skilled person would have known at the time that the patent application was filed.

21. I understand that the parties dispute the priority dates applicable to various claims of the Patents-in-Suit, ranging from May 29, 2003 to August 29, 2008. For the purposes of this Declaration, my opinions would not change using any of the priority dates in this range. Thus, for simplicity, I have been asked to conduct my analysis for the "mesylate" terms around or prior to the June 1, 2004 filing date of Application Nos. 10/857,619 and 10/858,526, to which the '486, '735, '254, '787, '788, '030, '031, '774, '770, '771, '466, '467, '936, '619, and '305 patents claim priority. I have also been asked to conduct my analysis for the "isolated" term around or prior to the May 29, 2003 filing date of Provisional Application No. 60/473,929, to which the '787 patent claims priority.

B. Person of Ordinary Skill in the Art

22. In conducting my analysis, I have considered and applied the understanding of a person of ordinary skill in the art ("POSA").

23. In my opinion, with respect to the Patents-in-Suit a POSA would be a person who at the relevant time held a Ph.D., or equivalent degree, in a field related to pharmaceutical sciences, and at least two years of experience in drug discovery and formulation, including in the development of potential drug candidates. The POSA may also have at least two years of experience in pharmaceutical formulation, analytical chemistry, pharmacology, pharmaceutics, crystallography, and/or the treatment of ADHD or consult with others having a Ph.D., Pharm.D., and/or M.D. degree and the requisite experience. I have used this definition of a POSA in forming my opinions contained herein.

24. I understand that Takeda has proposed defining a POSA as "[a] person with an academic degree of Doctor of Philosophy (or equivalent degree) in a field related to pharmaceutical sciences with approximately 1 year of relevant experience or a person with commensurate experience." (Joint Claim Construction and Prehearing Statement at 7.)

25. Takeda's definition is incomplete at least because it does not account for the fact that, in drug discovery and formulation, a POSA would consult with others within a particular area of expertise, as needed. Nevertheless, although I do

not agree with it, my opinions contained herein would not change using Takeda's definition of a POSA.

V. BACKGROUND OF THE TECHNOLOGY

A. Chemical Synthesis and Purification

26. Broadly speaking, nearly every process used to synthesize a chemical compound involves some sort of purification step. After a synthetic procedure, purification steps are used to remove contaminants, such as unreacted starting materials, other reagents, and side products. Such purification steps are important when synthesizing chemical compounds that can be used in pharmaceutical formulations because contaminants may be harmful or have unintended consequences.

27. Those skilled in the art would understand that there are a variety of steps that can be carried out to purify a compound. Common methods of purification include distillation, crystallization, extraction, and chromatography.

B. Salts

28. Salts are chemical species that contain ions (cations and anions) held together by attraction between positively charged ions (cations) and negatively charged ions (anions). This attraction is called Coulombic attraction. Solid pharmaceutical salts may, in addition to cations and anions, also include neutral molecules of either compound. When salts are dissolved, they generally dissociate into their constituent ions. For example, table salt is comprised of sodium cation

(Na^+) and chloride anion (Cl^-). As a solid, the ions bond together to form NaCl. When this salt is dissolved, it dissociates into positively charged sodium ions (Na^+) and negatively charged chlorine ions (Cl^-).

29. Salts of pharmaceutically active compounds are typically formed by reacting the parent or "free" form of the compound with either an acid or a base, depending on the properties of the parent. If the parent form of the compound is basic, it is reacted with an acid; if it is acidic, it is reacted with a base.

30. The ratio of the pharmaceutically active compound to counterions (e.g., mesylate) in a salt is often referred to as its stoichiometry. Where the components are present in equal amounts, the result is a 1:1 salt (pharmaceutically active compound:counterion). Other ratios include 2:1, 3:1, 1:2, 2:3, etc., as well as other non-whole number ratios.

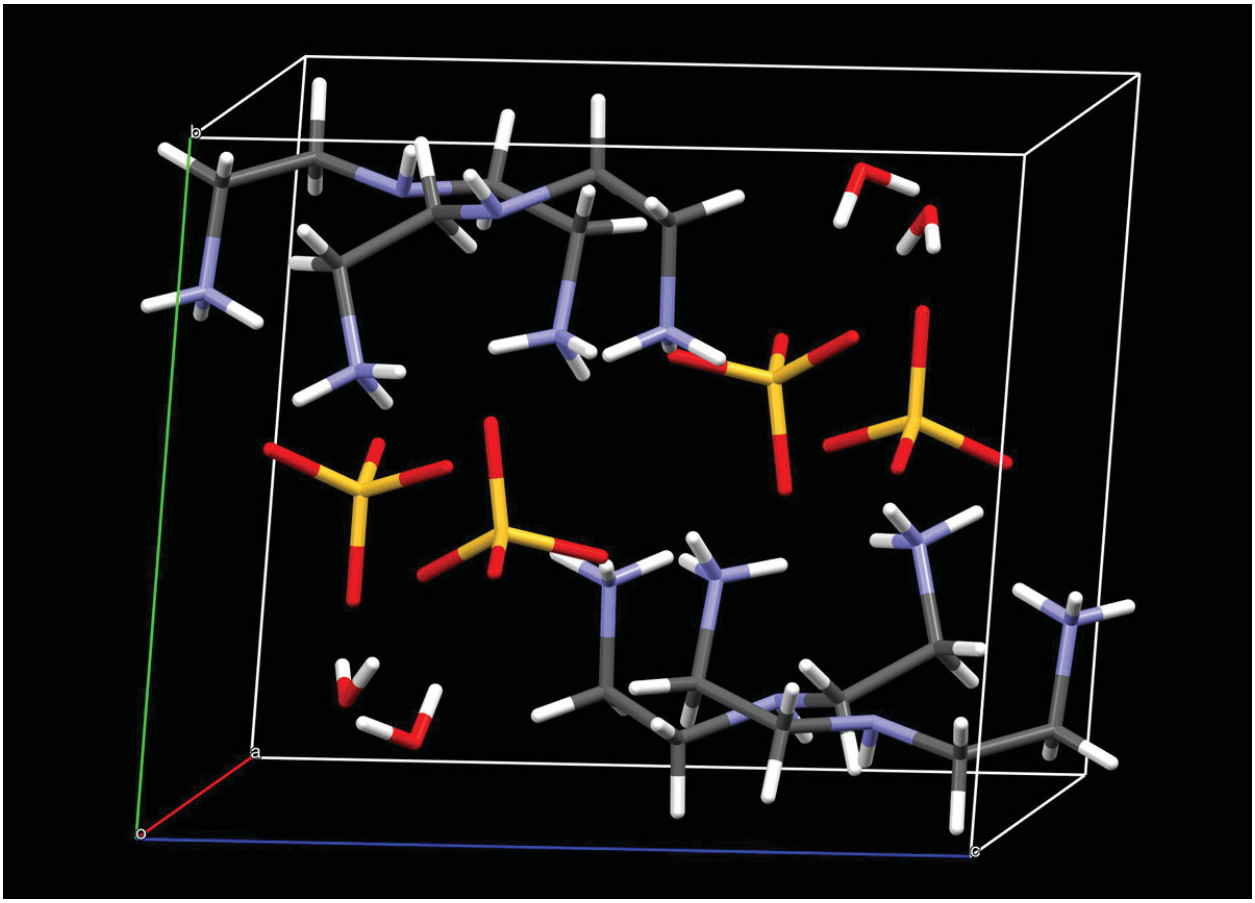
C. Solid Forms

31. A crystalline solid is a solid form in which the individual molecules or atoms are arranged or packed in a regularly repeating pattern with long-range order in three-dimensions. In contrast, an amorphous solid form (sometimes called a "glass") has considerable disorder in its structure and lacks the long-range order of a crystalline material.

32. The smallest repeating unit in a crystalline solid is the "unit cell," which represents the fundamental building block of that crystalline material. Each

unit cell is essentially a box with a specific size and shape that contains a particular number of molecules in a well-defined arrangement. The unit cell, as well as the arrangement of atoms within it, is the defining characteristic of a given crystalline form. When the crystalline form is a salt, the unit cell contains a particular number of acid and base molecules in a well-defined arrangement.

33. The following figure illustrates a unit cell of a crystalline salt of a non-pharmaceutical amine. Here, the components that are colored gray, blue, and white are the amine components. Those that are colored yellow and red are the counterions, whereas those that are colored red and white are water molecules.



VI. THE "MESYLATE" TERMS

34. It is my understanding that the "mesylate" terms appear in certain claims of fifteen of the Patents-in-Suit: the '486 patent (claim 4); the '735 patent (claim 3); the '254 patent (claim 3); the '787 patent (claim 3); the '788 patent (claims 5, 22, 27, 34, 41, 46); the '030 patent (claims 3, 10, 17, 24, 31, 38); the '031 patent (claims 6, 22); the '774 patent (claims 3, 10, 17, 24, 31, 38); the '770 patent (claim 4); the '771 patent (claims 3, 10, 17); the '466 patent (claim 10); the '467 patent (claims 3, 10, 17, 24, 31, 38); the '936 patent (claims 6, 22, 38, 54); the '619 patent (claims 3, 10, 17, 24, 31, 38); and the '305 patent (claims 3, 10, 17, 24, 31, 38).

35. Two of the "mesylate" terms recite the phrase "L-lysine-d-amphetamine mesylate." This phrase appears in the '735 patent (claim 3) and the '787 patent (claim 3). Claim 3 of the '787 patent is representative and is set forth below:

> 3. L-lysine-d-amphetamine mesylate.

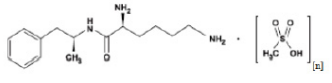
36. Three of the "mesylate" terms recite the phrase "mesylate salt of L-lysine-d-amphetamine." This phrase appears in the '486 patent (claim 4), the '254 patent (claim 3), and the '770 patent (claim 4). Claim 4 of the '486 patent is representative and is set forth below:

> 4. The method of 3, wherein the salt is a mesylate salt of L-lysine-d-amphetamine.

37. The remaining "mesylate" terms recite the phrase ". . . wherein said salt is a mesylate salt." This phrase appears in the '788 patent (claims 5, 22, 27, 34, 41, 46), the '030 patent (claims 3, 10, 17, 24, 31, 38), the '031 patent (claims 6, 22), the '774 patent (claims 3, 10, 17, 24, 31, 38), the '771 patent (claims 3, 10, 17), the '466 patent (claim 10), the '467 patent (claims 3, 10, 17, 24, 31, 38), the '936 patent (claims 6, 22, 38, 54), the '619 patent (claims 3, 10, 17, 24, 31, 38), and the '305 patent (claims 3, 10, 17, 24, 31, 38). Claim 3 of the '030 patent is representative and is set forth below:

> 3. A composition as defined in claim 2, wherein said salt is a mesylate salt.

38. I understand that the parties disagree on the meaning of the "mesylate" terms. I have been informed that Takeda and Norwich have proposed the following meanings for these terms:

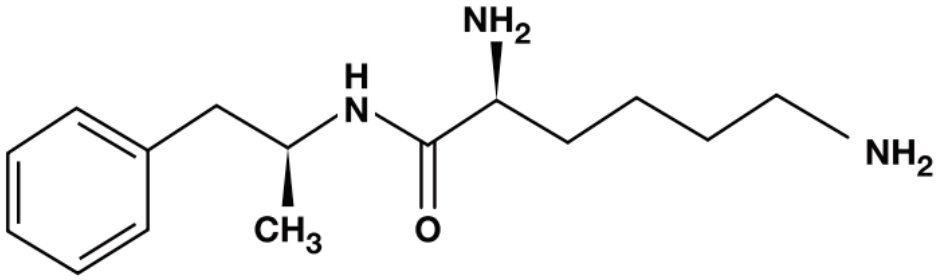
Claim Term	**Takeda's Proposed Construction**	**Norwich's Proposed Construction**
"L-lysine-d-amphetamine mesylate" *or* "mesylate salt of L-lysine-d-amphetamine" *or* "… wherein said salt is a mesylate salt"	a salt of L-lysine-d-amphetamine containing at least one CH3SO3- anion, which can be obtained from methanesulfonic acid (2S)-2,6-diamino-N-[(1S)-1-methyl-2-phenylethyl] hexanamide methanesulfonate 	"mesylate" / "a mesylate salt" means "a salt with any number of mesylate ions associated with it."

39. I have reviewed the ’486, ’735, ’254, ’787, ’788, ’030, ’031, ’774, ’770, ’771, ’466, ’467, ’936, ’619, and ’305 patents and their prosecution histories. While neither the “mesylate” terms nor the word “mesylate” itself are explicitly defined, in my opinion, these materials support Norwich’s proposed construction. In my opinion, the meaning of the “mesylate” terms is “a salt with any number of mesylate ions associated with it.”

40. For the purposes of understanding the “mesylate” terms, the specifications of the ’486, ’735, ’254, ’787, ’788, ’030, ’031, ’774, ’770, ’771, ’466, ’467, ’936, ’619, and ’305 patents are substantially the same. Therefore, I have generally referred to the specification of the ’486 patent unless otherwise indicated, although disclosures in the ’486 patent also apply to the other patents listed above.

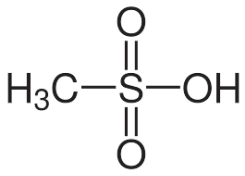
41. My understanding of the meaning of the “mesylate” terms is informed, in part, by the way in which these patents claim the “mesylate” salts of L-lysine-d-amphetamine. A POSA would understand that L-lysine-d-amphetamine has the chemical structure below.

L-lysine-d-amphetamine



42. Furthermore, a POSA would understand that "mesylate" is the name for the anion of methanesulfonic acid, which is depicted below.

Methanesulfonic Acid (CH_3SO_3H)



43. A POSA would understand that L-lysine-d-amphetamine is a basic compound due to the amine groups (NH_2) in its structure, and therefore could be converted to a cation during salt formation (i.e., accept a proton from another molecule).[1] A POSA would also understand that methanesulfonic acid is acidic due to the sulfonyl group ($-SO_3H$) in its structure, and therefore could act as an anion in salt formation (i.e., donate a proton (an ionized hydrogen atom) to another molecule).

[1] As discussed more fully below, neutral L-lysine-d-amphetamine may also be present in the mesylate salt.

44. Claim 3 of the '030 patent depends from claim 2, which, in turn, depends from claim 1. These claims are set forth below.

> 1. A composition comprising **an amount of from 25 to 75 mg of L-lysine-d-amphetamine or a salt thereof and having an amphetamine base amount of from 7.37 to 22.1 mg of said amphetamine**, said L-lysine-d-amphetamine or a salt thereof providing a mean $AUC_{0\text{-}12\ h}$ (ng h/mL) from 205.4±42.5 to 611.5±104.5, a mean AUC_{last} (ng h/mL) from 396.7±84.8 to 1237.0±194.0, a mean AUC_{inf} (ng h/mL) from 415.0±80.1 to 1259.5±191.3, a mean C_{max} (ng/mL) from 25.0±5.6 to 74.0±12.9, a mean T_{max} (hours) from 3.1±0.876 to 3.9±1.0, and a mean $T_{1/2}$ (hours) from 9.68±1.43 to 10.3±1.7 of amphetamine when orally administered to a human subject.
>
> 2. A composition as defined in claim 1, wherein said L-lysine-d-amphetamine is in the form of a salt.
>
> 3. A composition as defined in claim 2, wherein said salt is a mesylate salt.

As indicated above (with added emphasis), claim 1 recites "from 25 to 75 mg of L-lysine-d-amphetamine or a salt thereof and having an amphetamine base amount of from 7.37 to 22.1 mg of said amphetamine." Claim 2 narrows the term "L-lysine-d-amphetamine" such that it is "in the form of a salt," and claim 3 further narrows the salt such that "said salt is a mesylate salt." Thus, claim 3 of the '030 patent recites a composition comprising "an amount of from 25 to 75 mg of L-lysine-d-

amphetamine [mesylate] and having an amphetamine base amount of from 7.37 to 22.1 mg of said amphetamine."[2]

45. A POSA would understand that the amount of "amphetamine base" refers to the amount of amphetamine equivalent to a given amount of L-lysine-d-amphetamine. Thus, a POSA would understand that an amphetamine base amount from 7.37 to 22.1 mg is equivalent to 14.36 to 43.05 mg L-lysine-d-amphetamine (as a free base, i.e., when not in a salt form).[3]

46. Claim 3 also contemplates mesylate salts of L-lysine-d-amphetamine. These salts include those having a ratio of mesylate ions to L-lysine-d-amphetamine of at least 1:1. For example, a POSA would understand that claim 3 includes a salt containing 43.05 mg of L-lysine-d-amphetamine (equivalent to 22.1 mg of amphetamine base) in 75 mg of L-lysine-d-amphetamine mesylate salt, with

[2] This claim language is also present in the '030 patent (claim 24), the '774 patent (claims 3, 24), the '771 patent (claim 3), the '467 patent (claims 3, 24), the '619 patent (claims 3, 24), and the '305 patent (claims 3, 24). My analysis with respect to claim 3 of the '030 patent also applies to these other claims.

[3] A POSA would understand that the molecular weight of d-amphetamine is 135.21 mg/mmol and that the molecular weight of L-lysine-d-amphetamine is 263.3785 mg/mmol. Thus, (7.37 mg d-amphetamine base)×((263.3785 mg/mmol L-lysine-d-amphetamine)/(135.210 mg/mmol d-amphetamine base)) = 14.36 mg L-lysine-d-amphetamine. Similarly, (22.1 mg d-amphetamine base)×((263.3785 mg/mmol L-lysine-d-amphetamine)/(135.210 mg/mmol d-amphetamine base)) = 43.05 mg L-lysine-d-amphetamine.

the remaining 31.95 mg attributed to the mesylate component.[4] This salt has a ratio of 2:1 mesylate to L-lysine-d-amphetamine, corresponding to the dimesylate salt.

47. The claims could also encompass a monomesylate salt, which could be composed of 43.05 mg of L-lysine-d-amphetamine (equivalent to 22.1 mg amphetamine base) and 15.71 mg of mesylate ions for a total of 58.76 mg of L-lysine-d-amphetamine mesylate, which is within the claimed range of 25 to 75 mg of a pharmaceutically acceptable salt of L-lysine-d-amphetamine.[5]

48. But a POSA would also understand that claim 3 encompasses L-lysine-d-amphetamine mesylate salts that have ratios of mesylate ions to L-lysine-d-amphetamine that are less than 1:1. For example, a POSA would understand that claim 3 encompasses a salt containing 43.05 mg L-lysine-d-amphetamine and 7.85 mg mesylate for a total of 50.9 mg L-lysine-d-amphetamine mesylate. This

[4] Here, 43.05 mg L-lysine-d-amphetamine is equivalent to 0.16345 mmol, which is obtained by dividing the mass by the molecular weight (263.3785 mg/mmol). Further, 31.95 mg methanesulfonic acid is equivalent to 0.33245 mmol, which is again obtained by dividing the mass by the molecular weight (96.1057 g/mol). A POSA would understand that this corresponds to a molar ratio of 2.03:1 (~2:1), i.e., 0.33245 mmol methanesulfonic acid/0.16345 mmol L-lysine-d-amphetamine.

[5] A POSA would understand that the monomesylate salt contains equimolar amounts of L-lysine-d-amphetamine and mesylate. As indicated in footnote 4, 43.05 mg L-lysine-d-amphetamine equals 0.16345 mmol L-lysine-d-amphetamine. By the same logic, 15.71 mg of mesylate corresponds to 0.16345 mmol mesylate.

corresponds to a mesylate ion to L-lysine-d-amphetamine ratio 0.5:1.[6] And, because the amounts of L-lysine-d-amphetamine salt and equivalent amounts of amphetamine base are written as ranges, there are numerous other ratios of mesylate to L-lysine-d-amphetamine that are possible.

49. In view of these disclosures, a POSA would understand that the "mesylate" terms were neither restricted to a particular ratio (e.g., 2:1) nor restricted to a particular stoichiometry range. The claims, specifications, and prosecution histories do not suggest otherwise. Thus, a POSA would understand that the "mesylate" terms means "a salt with any number of mesylate ions associated with it."

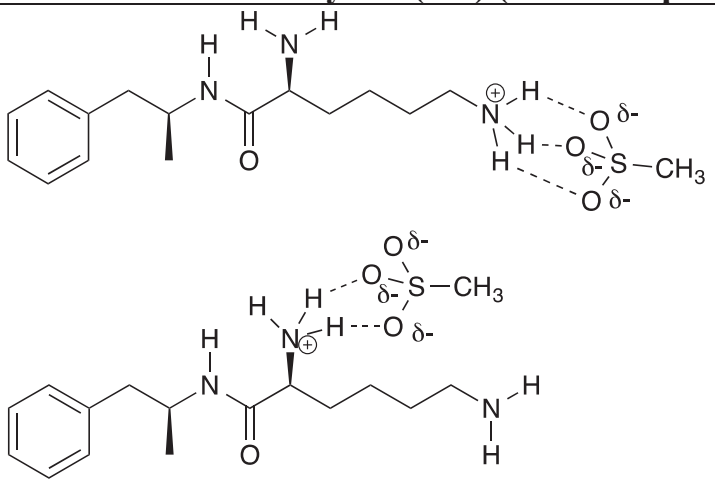
50. A POSA would also understand that this meaning of "mesylate" corresponded to its plain and ordinary meaning, which similarly has no restriction to a particular stoichiometry or range thereof. That is, a POSA would understand that a "mesylate" salt of L-lysine-d-amphetamine included salts containing all possible ratios of mesylate ions to L-lysine-d-amphetamine ions.

51. For example, a POSA would understand that a "mesylate" salt of L-lysine-d-amphetamine included a 1:1 ratio (monomesylate) and a 2:1 ratio

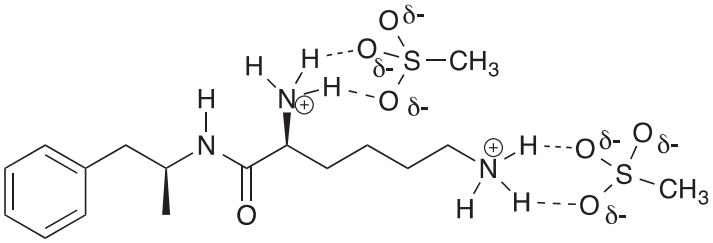
[6] By the logic in footnotes 4 and 5, a salt having a ratio of mesylate to L-lysine-d-amphetamine of 0.5:1 could be composed of 43.05 mg L-lysine-d-amphetamine (0.16345 mmol) and 7.85 mg of methanesulfonic acid (as mesylate ions; 0.08172 mmol), for a total mass of 50.9 mg L-lysine-d-amphetamine mesylate.

(dimesylate). A POSA would understand that these salts could occur, in part, because of the crystal packing pattern specific to each salt. Potential examples of crystal packing patterns for such crystals are illustrated in the examples below.

Potential Monomesylate (1:1) (two examples)

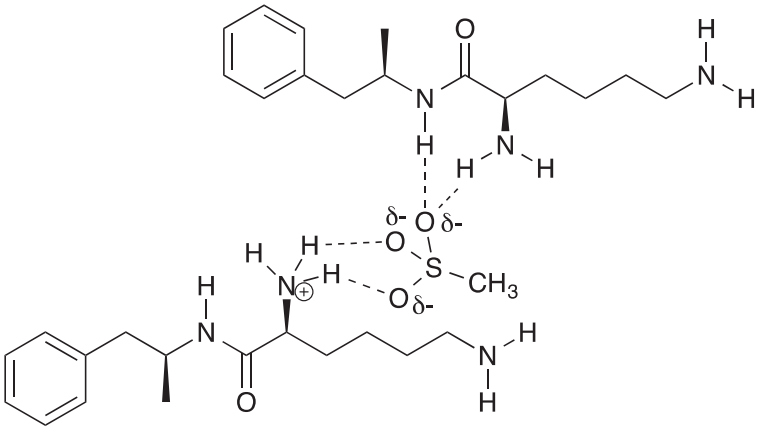


Potential Dimesylate (2:1)

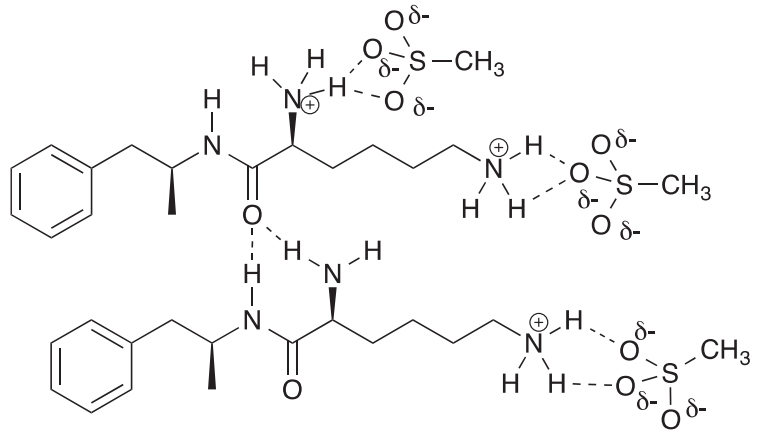


52. Similarly, a POSA would understand that a "mesylate" salt of L-lysine-d-amphetamine could include other ratios, and that salts with such ratios could again occur, in part, because of the crystal packing pattern specific to each salt, as illustrated below.

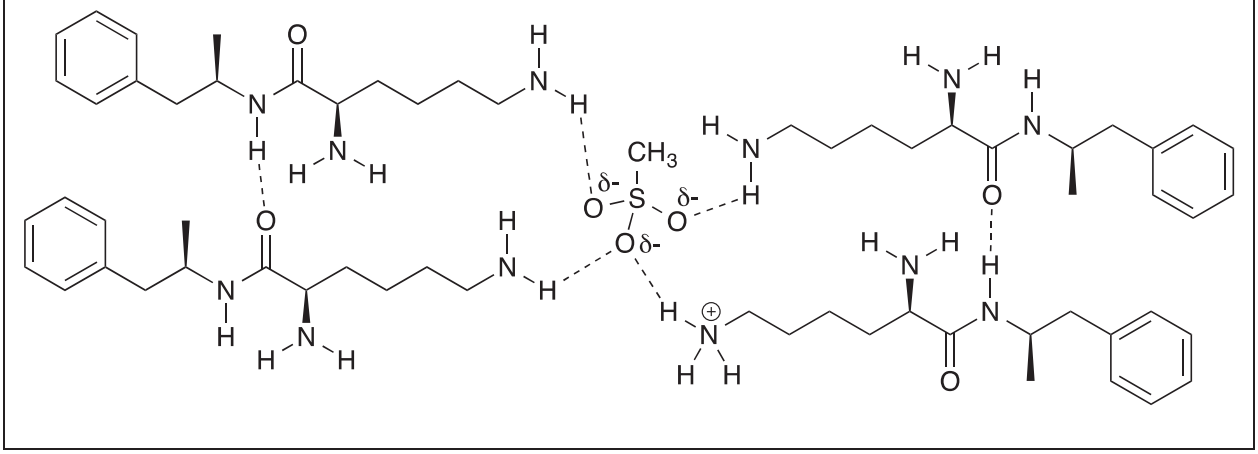
Potential 0.5:1 Ratio (1:2)



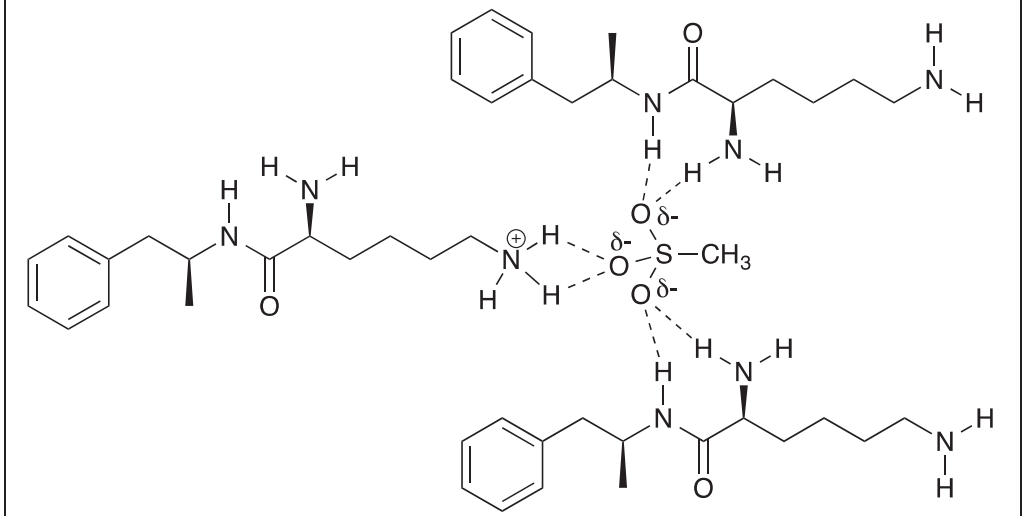
Potential 1.5:1 Ratio (3:2)



Potential 0.25:1 Ratio (1:4)



Potential 0.33:1 Ratio (1:3)



53. Furthermore, a POSA would understand that a "mesylate" salt of L-lysine-d-amphetamine included other ratios beyond those described above. Such ratios were known with other salts. *See, e.g.*, R. Rao Gadde et al., "High performance liquid chromatographic analysis of chlorhexidine phosphanilate, a new antimicrobial agent," *J. Pharm. & Biomed. Analysis*, **9**, 1031 (1991) at 1036

(disclosing the pharmaceutical salt chlorhexidine phosphanilate having a ratio of 1.83 phosphanilic acid to chlorhexidine) (attached as Exhibit C); Yong Guo & Aihua Huang, "A HILIC method for the analysis of tromethamine as the counter ion in an investigational pharmaceutical salt," *J. Pharm. & Biomed. Analysis*, **31**, 1191 (2003) at 1200 (disclosing various salts of an investigational drug compound with ratios of tromethamine to drug of 1.0, 1.8, 1.9, and 2.0) (attached as Exhibit D).

VII. THE "ISOLATED" TERM

54. It is my understanding that the "isolated" term appears in claims 1 and 2 of the '787 patent, which are set forth below.

> 1. A compound selected from the group consisting of isolated L-lysine-d-amphetamine and a pharmaceutically acceptable salt of L-lysine-d-amphetamine.
>
> 2. Isolated L-lysine-d-amphetamine.

55. I understand that the parties disagree on the meaning of the term "isolated." I have been informed that Takeda and Norwich have proposed the following meanings for this term:

Claim Term	Takeda's Proposed Construction	Norwich's Proposed Construction
"isolated"	"a substance separated from a crude mixture of reactants and/or solvents"	"non-salt form"

56. I have reviewed the '787 patent and its prosecution history. While the term "isolated" is not explicitly defined, in my opinion, these materials support Norwich's proposed construction. In my opinion, the meaning of "isolated" is "non-salt form."

57. My understanding of the meaning of the term "isolated" is informed, in part, by the structure of the claims of the '787 patent. The word "isolated" appears in two of the five claims of the '787 patent, all of which are set forth below.

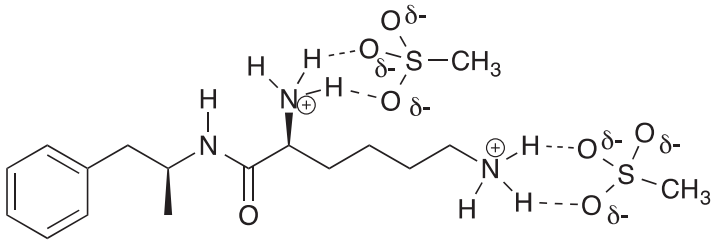
> 1. A compound selected from the group consisting of ***isolated*** L-lysine-d-amphetamine and a pharmaceutically acceptable salt of L-lysine-d-amphetamine.
>
> 2. ***Isolated*** L-lysine-d-amphetamine.
>
> 3. L-lysine-d-amphetamine mesylate.
>
> 4. L-lysine-d-amphetamine hydrochloride.
>
> 5. A pharmaceutically acceptable salt of L-lysine-d-amphetamine.

('787 patent, claims 1-5 (emphasis added).) As indicated above, claim 1 recites "[a] compound selected from the group consisting of" two options, which are "isolated L-lysine-d-amphetamine" and "a pharmaceutically acceptable salt of L-lysine-d-amphetamine."

58. A POSA would understand that the "isolated" term in claim 1 refers to the non-salt form of the compound, here L-lysine-d-amphetamine. He/she would

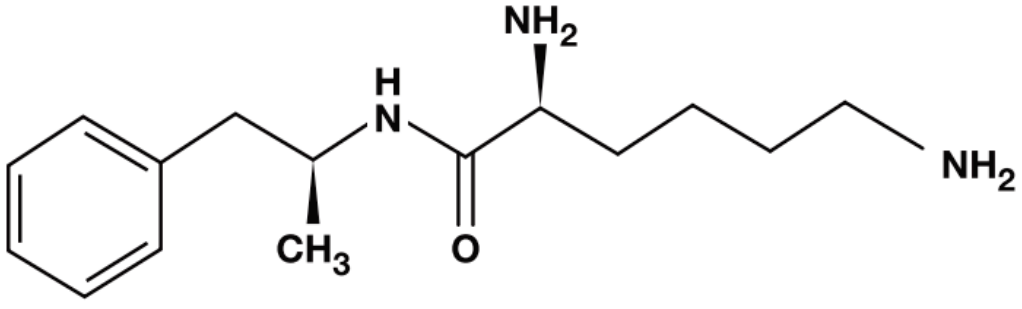
also understand that any pharmaceutically acceptable salt of L-lysine-d-amphetamine contained at least two components: the cation (L-lysine-d-amphetamine) and the anion (mesylate, for example).

Components of L-lysine-d-amphetamine dimesylate



Thus, a POSA would understand that L-lysine-d-amphetamine itself is not "isolated" when it is in the form of a salt. A POSA would also understand that, unlike a salt form, the non-salt form of L-lysine-d-amphetamine contains just one component: L-lysine-d-amphetamine (which is uncharged, and may be referred to as the free base).

L-lysine-d-amphetamine



Stated another way, a POSA would understand that claim 1 establishes a dichotomy between an "isolated" compound (having one component) and its pharmaceutically acceptable salts (having two or more components). If the term "isolated" included salts, that would make the second part of the group – pharmaceutically acceptable salts – superfluous.

59. The rest of the specification is consistent with my understanding. The word "isolated" only appears once in the specification, in describing the synthesis of L-lysine-d-amphetamine in Example 2. The full passage is set forth below, with the word "isolated" highlighted.

Example 2

Synthesis of L-lysine-d-amphetamine

L-lysine-d-amphetamine was synthesized (see FIG. **2**) by the following method:

a. Coupling

Reagents	MW	Weight	mmoles	Molar Equivalents
d-amphetamine freebase	135.2	4.75 g	35.13	1
Boc-Lys(Boc)-OSu	443.5	15.58 g	35.13	1
Di-iPr-Et-Amine	129	906 mg	7.03	0.2, d = 0.74, 1.22 mL
1,4-Dioxane	—	100 mL	—	—

To a solution of Boc-Lys(Boc)-OSu (15.58 g, 35.13 mmol) in dioxane (100 mL) under an inert atmosphere was added d-amphetamine freebase (4.75 g, 35.13 mmol) and DiPEA (0.9 g, 1.22 mL, 7.03 mmol). The resulting mixture was allowed to stir at room temperature overnight. Solvent and excess base were then removed using reduced pressure evaporation. The crude product was dissolved in ethyl acetate and loaded on to a flash column (7 cm wide, filled to 24 cm with silica) and eluted with ethyl acetate. The product was isolated; the solvent reduced by rotary evaporation and the

purified protected amide was dried by high-vac to obtain a white solid. ^{1}H NMR (DMSO-d_6) δ 1.02-1.11 (m, 2H, Lys γ-CH_2), δ 1.04 (d, 3H, Amp α-CH_3), δ 1.22-1.43 (m, 4H, Lys-β and δ-CH_2), δ 1.37 (18H, Boc, 6×CH_3), δ 2.60-2.72 (2H, Amp CH_2), δ 3.75-3.83, (m, 1H, Lys α-H) δ 3.9-4.1 (m, 1H, Amp α-H), δ 6.54-6.61 (d, 1H, amide NH), δ 6.7-6.77 (m, 1H, amide NH), δ 7.12-7.29 (m, 5H, ArH), δ 7.65-7.71 (m, 1, amide NH); mp=86-88° C.

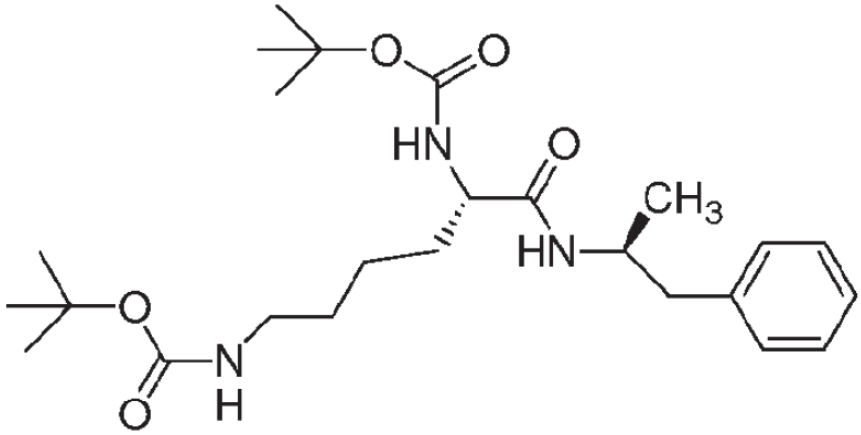
b. Deprotection

Reagents	MW	Weight	mmoles	Molar Equivalents
4M HCl in dioxane	4 mmol/mL	50 mL	200	6.25
Boc-Lys(Boc)-Amp	463.6	14.84 g	32	1
1,4-Dioxane	—	50 mL	—	—

The protected amide was dissolved in 50 mL of anhydrous dioxane and stirred while 50 mL (200 mmol) of 4M HCl/dioxane was added and stirred at room temperature overnight. The solvents were then reduced by rotary evaporation to afford a viscous oil. Addition of 100 mL MeOH followed by rotary evaporation resulted in a golden colored solid material that was further dried by storage at room temperature under high vacuum. ^{1}H NMR (DMSO-d_6) δ 0.86-1.16 (m, 2H, Lys γ-CH_2), δ 1.1 (d, 3H, Amp α-CH_3), δ 1.40-1.56 (m, 4H, Lys-β and δ-CH_2), δ 2.54-2.78 (m, 2H, Amp CH_2, 2H, Lys ϵ-CH_2), 3.63-3.74 (m, 1H, Lys α-H), δ 4.00-4.08 (m, 1H, Amp α-H), δ 7.12-7.31 (m, 5H, Amp ArH), δ 8.13-8.33 (d, 3H, Lys amine) δ 8.70-8.78 (d, 1H, amide NH); mp=120-122° C.

('787 patent at col. 20, l. 40 – col. 21, l. 31.)

60. As indicated above, Example 2 involves two steps. The first step, "coupling," involves attaching L-lysine to d-amphetamine. The result of this step is a product called Boc-Lys(Boc)-Amp. This is not a salt, but a precursor to L-lysine-d-amphetamine that includes two protecting groups (Boc) on the amine groups of the L-lysine moiety.



The specification states that this product is "isolated" before proceeding to the second step. The specification never states that a salt is "isolated."

61. In view of these disclosures, a POSA would understand that "isolated" L-lysine d-amphetamine means that the L-lysine d-amphetamine molecule itself is isolated from other molecules and atoms, including those that form salts with L-lysine d-amphetamine. Takeda's construction ("a substance separated from a crude mixture of reactants and/or solvents") fails to capture the distinction in the claims between "isolated L-lysine-d-amphetamine" and, on the other hand, its "pharmaceutically acceptable salt[s]."

VIII. CONCLUSION

62. For the reasons set forth above, it is my opinion that in the Patents-in-Suit, the "mesylate" terms mean "a salt with any number of mesylate ions associated with it" and that the term "isolated" means "non-salt form."

IX. SUPPLEMENTAL OPINIONS

63. If called to testify, my testimony may include an explanation of the principles that underlie the opinions expressed in this declaration.

64. I have based my opinion and analysis on documents and information available to me at the time I signed this declaration. If and when any new evidence arises, I reserve the right to supplement or modify my opinions to reflect that evidence.

I declare, under penalty of perjury, that I believe the foregoing is true and correct to the best of my knowledge and ability.

Dated: July 27, 2021

EXHIBIT A

EXHIBIT A

MATERIALS CONSIDERED BY MARK D. HOLLINGSWORTH, Ph.D.

Description
Joint Claim Construction and Prehearing Statement (dated June 24, 2021), specifically with respect to the "mesylate" and "isolated" terms
U.S. Patent No. 7,105,486 and its prosecution history
U.S. Patent No. 7,223,735 and its prosecution history
U.S. Patent No. 7,659,254 and its prosecution history
U.S. Patent No. 7,662,787 and its prosecution history
U.S. Patent No. 7,662,788 and its prosecution history
U.S. Patent No. 7,671,030 and its prosecution history
U.S. Patent No. 7,671,031 and its prosecution history
U.S. Patent No. 7,674,774 and its prosecution history
U.S. Patent No. 7,678,770 and its prosecution history
U.S. Patent No. 7,678,771 and its prosecution history
U.S. Patent No. 7,687,466 and its prosecution history
U.S. Patent No. 7,687,467 and its prosecution history
U.S. Patent No. 7,713,936 and its prosecution history
U.S. Patent No. 7,718,619 and its prosecution history
U.S. Patent No. 7,723,305 and its prosecution history
R. Rao Gadde et al., "High performance liquid chromatographic analysis of chlorhexidine phosphanilate, a new antimicrobial agent," *J. Pharm. & Biomed. Analysis*, **9**, 1031 (1991)
Yong Guo & Aihua Huang, A HILIC method for the analysis of tromethamine as the counter ion in an investigational pharmaceutical salt, *J. Pharm. & Biomed. Analysis*, **31**, 1191 (2003)

EXHIBIT B

CURRICULUM VITAE

MARK DAVID HOLLINGSWORTH
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EDUCATION:

Ph.D., ORGANIC CHEMISTRY (1986)
YALE UNIVERSITY, NEW HAVEN, CT. 06511

BACHELOR OF ARTS IN CHEMISTRY (1979)
CARLETON COLLEGE, NORTHFIELD, MN 55057

FELLOWSHIPS AND AWARDS:

ALFRED P. SLOAN RESEARCH FELLOWSHIP (1991-3)

DISTINGUISHED DISSERTATION AWARD (IN PHYSICAL SCIENCES AND ENGINEERING, 1983-1987)
NORTHEASTERN ASSOCIATION OF GRADUATE SCHOOLS

1987 NOBEL LAUREATE SIGNATURE AWARD FOR GRADUATE EDUCATION IN CHEMISTRY (AMERICAN CHEMICAL SOCIETY)

RICHARD WOLFGANG MEMORIAL PRIZE
YALE UNIVERSITY (MAY, 1986)

NSF-NATO POSTDOCTORAL FELLOWSHIP
UNIV. OF CAMBRIDGE (1986-1987)

S.E.R.C. POSTDOCTORAL RESEARCH ASSISTANTSHIP
UNIV. OF CAMBRIDGE (1985-1986)

DOX FELLOWSHIP FOR RESEARCH IN ORGANIC CHEMISTRY
YALE UNIVERSITY (SUMMER, 1983)

MEMBERSHIPS:

SIGMA XI
KING'S COLLEGE, CAMBRIDGE
AMERICAN CHEMICAL SOCIETY
AMERICAN CRYSTALLOGRAPHIC ASSOCIATION
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

POSITIONS AND RESEARCH EXPERIENCE:

6-2006 TO 7-2006	VISITING PROFESSOR, DEPT. OF CHEMISTRY, UNIV. BORDEAUX
7-01, 7-02, 3-05, 6-06, 5-07, 10-08, 6-09, 5-10, 6-12, 6-14	VISITING PROFESSOR, DEPT. OF PHYSICS, UNIV. OF RENNES
5-18, 9-18	CNRS VISITING ROFESSOR, DEPT. OF PHYSICS, UNIV. OF RENNES
8-98 TO PRESENT	ASSOC. PROF., CHEMISTRY DEPT., KANSAS STATE UNIVERSITY
9-91 TO 8-98	ASST. PROF., CHEMISTRY DEPT., INDIANA UNIVERSITY
10-87 TO 8-91	ASST. PROF., CHEMISTRY DEPT., UNIVERSITY OF ALBERTA
11-85 TO 8-87	POSTDOCTORAL RESEARCH ASSISTANT AND FELLOW DEPT. OF PHYSICAL CHEMISTRY, UNIV. OF CAMBRIDGE
5-80 TO 10-85	GRADUATE STUDENT, CHEMISTRY DEPT., YALE UNIVERSITY
1-78 TO 3-79	UNDERGRADUATE, CARLETON COLLEGE

RESEARCH EXPERIENCE

CURRENT WORK AT KANSAS STATE UNIVERSITY
Research in solid-state organic chemistry with emphasis on dynamic and structural phenomena and on cooperative interactions in solid-state processes. During recent years, this work has focused on series of ferroelastic and ferroelectric inclusion compounds and salts that we have developed and on the generation of a scale of functional group interaction energies through the measurement of solid-state NMR spectra of inclusion compounds. Our work includes mechanistic studies of crystal growth and domain switching in organic single crystals and on phase transitions, and it emphasizes crystal engineering, microscopy, spectroscopy and crystallography of organic solids with specific chemical and physical properties. Much of our most recent work has involved the physical crystallography of aperiodic crystals.

UNIVERSITY OF CAMBRIDGE, 11-85 TO 8-87 (POSTDOCTORAL)
Research in solid-state photochemistry emphasizing structural and dynamic characterization of reactive intermediates generated from organic molecules trapped in zeolites and inclusion compounds. I used radical pair EPR spectroscopy to study the effects of long-range stress and intra- and intermolecular substituents on solid-state reactions, but I also worked solid-state photochemistry/EPR spectroscopy of acridizinium halides and X-ray crystallography.

YALE UNIVERSITY, 1980-1985 (GRADUATE)
Dissertation: Infrared Studies of CO_2 Dimers as a Probe of Local Stress in Solid State Peroxide Reactions. In this research I used the asymmetric stretching mode of CO_2 to study the structure and dynamics of CO_2 dimers photogenerated in single crystals of diacyl peroxides. This work demonstrates that motion of the intermediates is controlled by anisotropic stresses equivalent to 20-30 kbar or more. Polarized IR studies of oriented crystals and analysis of intermolecular resonant coupling were used to determine the orientations of the CO_2 molecules, while substituent and intra- and intermolecular isotope effects helped elucidate the reaction pathways.

Single crystal radical pair ESR of the ground state of the benzoyloxyl radical: This study, which built upon earlier work in our group on the ^{17}O-hyperfine anisotropy of the benzoyloxyl radical, involved measurement of the ^{13}C hfs constants of this radical in ^{13}C-1-labeled acetyl benzoyl peroxide. The ^{13}C hfs constants corroborated the earlier claim of a $^2B_2$ ground state.

Synthesis directed toward ESR studies of the norbornenyl-nortricyclyl radical rearrangement in the solid state: This project involved design and synthesis of compounds that yield spectroscopically suitable radical pairs upon photolysis.

My dissertation work was recognized with the Wolfgang Memorial Prize (best chemistry dissertation at Yale), the 1987 Nobel Laureate Signature Award for Graduate Education in Chemistry (best chemistry dissertation in the U.S.) and the Distinguished Dissertation Award from the Northeastern Association of Graduate Schools (most distinguished dissertation in physical sciences and engineering from 1983-1987 in a consortium of approximately 60 graduate schools in the northeastern United States.)

CARLETON COLLEGE, 1978-79 (UNDERGRADUATE)

With two Carleton professors, C.E. Buchwald (geologist) and J.R. Mohrig (chemist) I conducted a yearlong study of the Cannon River at Northfield, Minn. I was mainly concerned with very accurate measurement of the river's dissolved oxygen content, to see if the local sewage treatment plant's methods were in any way deficient. I also conducted organic chemistry research with Prof. Nancy S. Mills on "Y-aromaticity" of the dianion of 2-methyl- 2-butene. This research included synthesis, quenching and NMR spectroscopy of these dianions.

PUBLICATIONS:

50. B. Toudic, L. Guérin, C. Mariette, I. Frantsuzov, P. Rabiller, C. Ecolivet, and Mark D. Hollingsworth, "Comment on Couzi *et al*. (2018): A Phenomenological Model for Structural Transitions in Incommensurate Alkane/urea Inclusion Compounds," *R. Soc. Open Sci*., 6, 182073 (2019). DOI: 10.1098/rsos.182073.

49. B. Kahr, L. B. McHenry, and M. D. Hollingsworth, "Academic Publishing and Scientific Integrity: Case Studies of Editorial Interference at Taylor & Francis," *J. Sci. Pract. Integ*., 1, final pages not yet assigned (2019). DOI: 10.35122/jospi.2019.740579.

48. B. Kahr and M. D. Hollingsworth, "Massive Faculty Donations and Institutional Conflicts of Interest," *J. Sci. Pract. Integ*., 1 (2019). DOI: 10.35122/jospi.2019.848394.

47. B. Toudic, L. Guérin, C. Mariette, I. Frantsuzov, P. Rabiller, C. Ecolivet, T. Janssen, and M. D. Hollingsworth, "Comment on "The True Structural Periodicities and Superspace Group Descriptions of the Prototypical Incommensurate Composite Materials: Alkane/urea Inclusion Compounds," by Couzi M. et al.," *EPL*, **119**, 66004 (2017); DOI: 10.1209/0295-5075/119/66004. This paper was selected by the Editor in Chief to be open access.

46. C. Mariette, I. Frantsuzov, B. Wang, L. Guérin, P. Rabiller, M. D. Hollingsworth, and B. Toudic, "Frustrated Pretransitional Phenomena in Aperiodic Composites," *Phys. Rev. B*., **94**, 184105 (2016); DOI: 10.1103/PhysRevB.94.184105.

45. L. Guérin, C. Mariette, P. Rabiller, M. Huard, S. Ravy, P. Fertey, Shane M. Nichols, Bo Wang, Stefan C. B. Mannsfeld, T. Weber, Mark D. Hollingsworth, and B. Toudic, "Long-range Modulation of a Composite Crystal in a Five-dimensional Superspace," *Phys. Rev. B*, **91**, 184101 (2015); DOI: 10.1103/PhysRevB.91.184101.

44. C. Mariette, L. Guérin, P. Rabiller, Y.-S. Chen, A. Bosak, A. Popov, M. D. Hollingsworth, and B. Toudic, "The Creation of Modulated Monoclinic Aperiodic Composites in *n*-Alkane/urea Compounds," *Z. Kristallogr*., **230**, 5-11 (2015); DOI: 10.1515/zkri-2014-1773.

43. P. Rabiller, B. Toudic, C. Mariette, L. Guérin, C. Ecolivet, M. D. Hollingsworth, "Phase Transitions in Aperiodic Crystals," in *Aperiodic Crystals*, Siegbert Schmid, Ray L. Withers, Ron Lifshutz, Eds., Springer Science+Business Media, Dordrecht, 2013, pp. 171-177; DOI: 10.1007/978-94-007-6431-6.

42. C. Mariette, L. Guérin, P. Rabiller, C. Ecolivet, P. García-Orduña, P. Bourges, A. Bosak, D. de Sanctis, M. D. Hollingsworth, T. Janssen, B. Toudic, "Critical Phenomena in Higher Dimensional Spaces: The Hexagonal-to-Orthorhombic Phase Transition in Aperiodic *n*-Nonadecane/urea," *Phys. Rev. B,* **87**, 101401 (2013); DOI: 10.1103/PhysRevB.87.104101

41. M. D. Hollingsworth, M. L. Peterson, B. D. Dinkelmeyer, "Space Group Assignment and Evaluation of End-For-End Guest Disorder in Urea Inclusion Compounds," *Trans. Am. Cryst. Assn*., **43**, 113-127 (2012).

40. C. Mariette, M. Huard, P. Rabiller, S. M. Nichols, C. Ecolivet, T. Janssen, K. E. Alquist, III, M. D. Hollingsworth, B. Toudic, "A Molecular "Phase-Ordering" Phase Transition leading to a Modulated Aperiodic Composite in *n*-Heptane/urea," *J. Chem. Phys.,* **136**, 104507-1 to 104507-6 (2012); DOI: 10.1063/1.3692329

39. M. Huard, B. Toudic, P. Rabiller, C. Ecolivet, P. Bourges, T. Breczewski, M. D. Hollingsworth, "Confined Linear Molecules Inside an Aperiodic Supramolecular Crystal: the Sequence of Superspace Phases in *n*-Hexadecane/urea," *J. Chem. Phys.,* **135**, 204505-1 to 204505-8 (2011); DOI: 10.1063/1.3663711

38. M. D. Hollingsworth, "Calcite Biocomposites Up Close," an invited Perspective for *Science*, **326**, 1194-1195 (Nov. 27, 2009); DOI: 10.1126/science.1183122

37. B. Toudic, P. Garcia, C. Odin, P. Rabiller, C. Ecolivet, E. Collet, P. Bourges, G. J. McIntyre, M. D. Hollingsworth, T. Breczewski, "Hidden Degrees of Freedom in Aperiodic Materials," *Science*, **319**, 69-71 (Jan. 4 2008). DOI: 10.1126/science.1146745. See also P. Coppens, "A Phase Transition Hidden in Higher Dimensions (*Science*, **319**, 41-42, Jan. 4, 2008) and S. Hurtley and P. Szuromi "Motion in a Higher Plane," (*Science*, **319**, 11, Jan. 4, 2008).

36. M. D. Hollingsworth, "Molecular Recognition within One-dimensional Channels," in *Turning Points in Solid-state, Materials and Surface Science: A Book in Celebration of the Life and Work of Sir John Meurig Thomas*," K. D. M Harris, P. P. Edwards, Eds., The Royal Society of Chemistry, Cambridge, 2007, pp. 346-361. DOI: 10.1039/9781847558183-00346.

35. M. D. Hollingsworth, J. A. Swift, B. Kahr, "J. Michael McBride at 65 - An Appreciation," *Cryst. Growth Des.*, **5**, 2022-2035 (2005). A Perspective with 103 references for the Special Issue in honor of J. Michael McBride. DOI: 10.1021/cg0505362.

34. M. D. Hollingsworth, M. L. Peterson, J. R. Rush, M. E. Brown, M. J. Abel, A. A. Black, M. Dudley, B. Raghothamachar, U. Werner-Zwanziger, E. J. Still, J. A. Vanecko, "Memory and Perfection in Ferroelastic Inclusion Compounds," *Cryst. Growth Des.*, **5**, 2100-2116 (2005). DOI: 10.1021/cg050347j.

33. M. D. Hollingsworth and M. L. Peterson, "Twinning, Epitaxy, and Domain Switching in Ferroelastic Inclusion Compounds," *Proceedings of the NASA Microgravity Materials Science Conference 2002*, D. Gillies, N. Ramachandran, K. Murphy, D. McCauley, N. Bennett, eds., 283-288, Feb. 2003.

32. M. D. Hollingsworth, "Crystal Engineering: from Structure to Function," an invited Viewpoint for *Science*, **295**, 2410-2413 (March 29, 2002). DOI: 10.1126/science.1070967. (See also the cover of this issue.)

31. M. D. Hollingsworth, M. E. Brown, M. Dudley, H. Chung, M. L. Peterson and A. C. Hillier,

"Template Effects, Asymmetry and Twinning in Helical Inclusion Compounds," *Angew. Chem. Int. Ed.*, **41**, 965-969 (2002). DOI: 10.1002/1521-3757(20020315)114:6<1007::AID-ANGE1007>3.0.CO;2-9.

30. M. D. Hollingsworth, M. L. Peterson, K. L. Pate, B. Dinkelmeyer and M. E. Brown, "Unanticipated Guest Motion During a Phase Transition in a Ferroelastic Inclusion Compound," *J. Am. Chem. Soc.* **124**, 2094-2095 (2002). DOI: 10.1021/ja010327f.

29. M. D. Hollingsworth, U.Werner-Zwanziger, M. E. Brown, J. D. Chaney, J. C. Huffman, K. D. M. Harris and S. P. Smart, "Spring-loading at the Molecular Level: Relaxation of Guest-Induced Strain in Channel Inclusion Compounds," *J. Am. Chem. Soc.*, **121**, 9732-9733 (1999). DOI: 10.1021/ja9919534.

28. U. Werner-Zwanziger, M. E. Brown, J. D. Chaney, E. J. Still, M. D. Hollingsworth, "Deuterium NMR Studies of Guest Motions in Urea Inclusion Compounds of 1,6-Dibromohexane with Analytical Evaluation of Spectra in the Fast Motion Limit," *Appl. Magn. Reson.*, 17(2-3), 265-281 (1999). DOI: 10.1007/BF03162165.

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26. P. Girard, A. E. Aliev, F. Guillaume, K. D. M. Harris, M. D. Hollingsworth, A.-J. Dianoux, P. Jonsen, "Reorientational Motions of Dioctanoyl Peroxide Guest Molecules within the Urea Tunnel Structure: Assessment of Two-site Jump Models," *Physica B* (Amsterdam), **234-236**, 112-114 (1997). DOI: 10.1016/S0921-4526(96)00900-3.

25. J. D. Chaney, C. R. Goss, K. Folting, B. D. Santarsiero and M. D. Hollingsworth, "Formyl C-H¨¨O Hydrogen Bonding in Crystalline Bis-Formamides?" *J. Am. Chem. Soc.*, **118**, 9432-3 (1996). DOI: 10.1021/ja960637b.

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23. M. E. Brown, J. D. Chaney, B. D. Santarsiero, M. D. Hollingsworth, "Superstructure Topologies and Host-Guest Interactions in Commensurate Inclusion Compounds of Urea with Bis(methylketone)s," *Chem. Mater.*, **8**, 1588-1591 (1996). DOI: 10.1021/cm960264d.

22. M. D. Hollingsworth, "Inclusion Compounds," *Current Opinion in Solid State and Materials Science*, **1**, 514-521 (1996). DOI: 10.1016/S1359-0286(96)80067-2. Invited review concerning the state of the field.

21. H. Chung, M. Dudley, M. E. Brown and M. D. Hollingsworth, "Synchrotron White Beam X-ray Topography Characterization of Defect Structures in 2,10-Undecanedione/Urea Inclusion Compounds," *Mol. Cryst. Liq. Cryst.*, **276**, 203-212 (1996). DOI: 10.1080/10587259608039378.

20. M. D. Hollingsworth and K. D. M. Harris, "Urea, Thiourea and Selenourea," *Comprehensive Supramolecular Chemistry* (Vol. 6; Solid State Supramolecular Chemistry: Crystal Engineering), Atwood, J. L., Davies, J. E. D., MacNicol, D. D. and Vogtle, F., eds., pp. 177-237 (1996).

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18. M. E. Brown, M. D. Hollingsworth and B. D. Santarsiero, "Small Molecule Diffraction Studies with the R-Axis Area Detector - Structural Aspects of a Class of Urea Inclusion Compounds," *The Rigaku Journal*, **11**, 4-8 (1994).

17. M. D. Hollingsworth, M. E. Brown, B. D. Santarsiero, J. C. Huffman and C. R. Goss, "Template Directed Synthesis of 1:1 Layered Complexes of α,ω-Dinitriles and Urea: Packing Efficiency versus Specific Functional Group Interactions," *Chem. Mater.*, **6**, 1227-1244 (1994). DOI: 10.1021/cm00044a022.

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11. K. D. M. Harris and M. D. Hollingsworth, "Structural Properties of the Guest Species in Diacyl Peroxide/Urea Inclusion Compounds: An X-ray Diffraction Investigation," *Proc. Roy. Soc. London A,* **431**, 245-269 (1990). DOI: 10.1098/rspa.1990.0129.

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9. M. D. Hollingsworth and N. Cyr, "High Resolution Solid-State NMR Spectra of Leucine: A Re-examination," *J. Chem. Soc. Chem. Commun.*, 578-80, 1990. DOI: 10.1039/C39900000578.

8. M. D. Hollingsworth and J. M. McBride, "Photochemical Mechanism in Single Crystals: FTIR Studies of Diacyl Peroxides," *Advances in Photochemistry*, Vol. 15, D. Volman, G. Hammond, K. Gollnick, Eds., John Wiley and Sons, Inc. (1990), pp. 279-379. DOI: 10.1002/9780470133453.ch5.

7. K. D. M. Harris and M. D. Hollingsworth, "Losing Symmetry by Design," *Nature*, **341**, 19 (Sept. 7, 1989). DOI: 10.1038/341019a0. Invited News and Views article.

6. M. D. Hollingsworth and J. M. McBride, "Infrared Studies of Long-Range Stress in Solid-State Peroxide Photoreactions," *Mol. Cryst. Liq. Cryst. Inc. Nonlin. Opt.*, **161**, 25-41 (1988). DOI: 10.1080/00268948808070237.

5. M. D. Hollingsworth, K. D. M. Harris, W. Jones and J. M. Thomas, "ESR and X-ray Diffraction Studies of Diacyl Peroxides in Urea and Aluminosilicate Hosts," *J. Inclus. Phenom.*, **5**, 273-277 (1987). DOI: 10.1007/BF00655664.

4. M. D. Hollingsworth and J. M. McBride, "Coupling of CO_2 Asymmetric Stretching in Dimers Photogenerated Within Long-Chain Diacyl Peroxide Single Crystals," *Chem. Phys. Lett.*, **130**, 259-264 (1986). DOI: 10.1016/0009-2614(86)80466-3.

3. J. M. McBride, B. E. Segmuller, M. D. Hollingsworth, D. E. Mills and B. A. Weber, "Mechanical Stress and Reactivity in Organic Solids," *Science*, **234**, 830-835 (1986). DOI: 10.1126/science.234.4778.830. (Research Article)

2. M. D. Hollingsworth and J. M. McBride, "Specific Long-Range Effects on Relaxation of Local Stress during a Solid-State Reaction," *J. Am. Chem. Soc.*, **107**, 1792-1793 (1985). DOI: 10.1021/ja00292a073.

1. N. S. Mills, J. Shapiro and M. D. Hollingsworth, "Dianions of 2-Methyl-2-butene: Evidence for the Stability of a "Y-Aromatic" Species," *J. Am. Chem. Soc.*, **103**, 1263-1264 (1981). DOI: 10.1021/ja00395a066.

OTHER PUBLICATIONS

B. Kahr and M. D. Hollingsworth, "Misconduct Investigators, Show Your Work," invited Comment for Chemistry World, April 2019.

M. D. Hollingsworth and M. D. Ward, "Margaret Cairns (Peggy) Etter 1943-1992," *Chem. Mater.*, **6**, 1087-1089 (1994). DOI: 10.1021/cm00044a001.

M. D. Ward and M. D. Hollingsworth, "Preface to the Special Issue," *Chem. Mater.*, **6**, 1093 (1994). DOI: 10.1021/cm00044a600.

M. D. Hollingsworth, "Triphosgene Warning," Chemical and Engineering News, July 13, 1992, p. 4 - letter to the editor.

M. D. Hollingsworth, "Infrared Studies of CO_2 Dimers as a Probe of Stress in Solid State Peroxide Reactions," Ph. D. Dissertation, Yale University, Nov. 1985, 1106 pp.

CONTRIBUTIONS TO CONFERENCES AND SYMPOSIA

87. Invited Lecture: M. D. Hollingsworth, B. Wang, I. Frantsuzov, S. M. Nichols, P. Rabiller, C. Mariette, L. Guérin, and B. Toudic, "Phase Transitions in Organic Inclusion Compounds," 24th International Conference on the Chemistry of the Organic Solid State, New York, NY, June 16-21, 2019.

86. Invited Lecture: M. D. Hollingsworth, "Phase transitions and self-compression in channel inclusion compounds," workshop on Breaking and Making Bonds with Light, Telluride, CO July 9-12, 2018.

85. Invited Plenary Lecture: M. D. Hollingsworth, Bo Wang, Ilya S. Frantsuzov, Shane M. Nichols, Philippe Rabiller, Céline Mariette, Laurent Guérin, and Bertrand Toudic, "Self-compression in Channel Inclusion Compounds," 23rd International Conference on the Chemistry of the Organic Solid State, Stellenbosch, South Africa, April 2-7, 2017.

84. Invited Lecture: M. D. Hollingsworth, "Compression and Self-compression in Channel Inclusion Compounds," workshop on Energy and Movement in Coherent Chemical Systems, Telluride, CO July 4-8, 2016.

83. Invited Lecture: M. D. Hollingsworth, B. Wang, I. S. Frantsuzov, S. M. Nichols, P. Rabiller, C. Mariette, L. Guérin, and B. Toudic, "Synchrotron studies of self-compression in channel inclusion compounds," Pacifichem 2015, Honolulu, HI, Dec. 2015.

82. Invited Lecture: M. D. Hollingsworth, "The Midwest Organic Solid State Chemistry Symposium: Origins and the Early Years," 24th Midwest Organic Solid State Chemistry Symposium, Iowa City, IA, June, 2014.

81. Invited Lecture: M. D. Hollingsworth, B. Wang, S. M. Nichols, X. Li, P. Rabiller, B. Toudic, "Synchrotron Studies of Phase Transitions in Channel Inclusion Compounds," 2013 Midwest Regional Meeting of the American Chemical Society, Springfield, MO, Oct. 16-18, 2013.

80. Contributed Lecture: M. D. Hollingsworth, B. Wang, S. M. Nichols, X. Li, B. Toudic, P. Rabiller, C. Mariette, M. Huard, and L. Guerin, "Crystal growth and phase transitions in commensurate and incommensurate inclusion compounds," 21st International Conference on the Chemistry of the Organic Solid State, Oxford, United Kingdom, Aug. 4-9, 2013.

79. Invited Lecture: M. D. Hollingsworth, "Surface Roughening, Molecular Recognition, and Phase Transitions in Channel Inclusion Compounds," Symposium in Honor of J. Michael McBride, Department of Chemistry, Yale University, New Haven, Oct. 27, 2012

78. Invited Lecture: M. D. Hollingsworth S. M. Nichols, B. Wang, K. E. Alquist III, A. D. Adams, B. Toudic, P. Rabiller, M. Huard, C. Mariette, and L. Guerin, "Channel Inclusion Compounds in Three, Four, and Five Dimensions," Transactions Symposium, American Crystallographic Association National Meeting, Boston, MA, July 2012.

77. Invited Lecture: M. D. Hollingsworth, B. Wang, S. M. Nichols, B. Toudic, P. Rabiller, "Substituent Effects and Domain Switching in Periodic and Aperiodic Inclusion Compounds," Colloque d'Agence Nationale de la Recherche à Rennes: Stability of Aperiodic Phases, University of Rennes, Rennes, France, July 2-3, 2012.

76. Contributed lecture: M. D. Hollingsworth, J. Bacsa, J. R. Rush, K. E. Alquist III, S. M. Nichols, B. Toudic, P. Rabiller, "Surface Roughening and Crystal Growth of Channel Inclusion Compounds," 2011 American Conference on Crystal Growth and Epitaxy, Monterey, CA, July 31-August 5, 2011.

75. Invited lecture: M. D. Hollingsworth; F. Nozirov; R. B. Gajda; S. M. Nichols; E. J. Chan; W. Kaminsky, "Structures and Dynamics of Organic Ferroelastics and Ferroelectrics," Pacifichem 2010, Honolulu, Hawaii, Dec. 14-18, 2010.

74. Invited Lecture: M. D. Hollingsworth, S. M. Nichols, F. Nozirov, R. B. Gajda, E. J. Chan, M. Huard, B. Toudic, P. Rabiller, C. Ecolivet, and C. Mariette, "Stress and Strain in Crystal Engineering," 5th Bologna Convention on Crystal Forms, Bologna, Italy, Sept. 2-4, 2010.

73. Invited Lecture: M. D. Hollingsworth, J. Bacsa, J. R. Rush, K. E. Alquist III, S. M. Nichols, B. Toudic, P. Rabiller, "Surface Roughening in the Crystal Growth of Channel Inclusion Compounds," 19th International Conference on the Chemistry of the Organic Solid State, Sestri Levante, Italy, June 14-19, 2009.

72. Contributed Lecture: M. D. Hollingsworth, M. L. Peterson, D. S. Kesselring, A. G. Butenhoff, D. A. Higgins, G. Springer, F. Guillaume, "A New Family of Ferroelastic and Ferroelectric Calixarenes," Nineteenth Midwest Organic Solid State Chemistry Symposium, Manhattan, KS, June 2008.

71. Invited Lecture/Panel Discussion: M. D. Hollingsworth, "Design and Control of Structure and Function of Molecular Crystals and Solid-State Supramolecular Assemblies," Chemistry for the 21st Century, Yale Alumni Chemistry Reunion, Yale University, Nov. 2-4, 2007.

70. Invited Lecture: M. D. Hollingsworth, M. L. Peterson, J. R. Rush, M. J. Abel, A. A. Black, "Memory and Perfection in Ferroelastic Inclusion Compounds," 42nd Congress of the Mexican Chemical Society, Guadalahara, Mexico, Sept. 2007.

69. Plenary Lecture: M. D. Hollingsworth, J. R. Rush, J. Bacsa, F. Guillaume, A. Desmedt, M. J. Abel, and A. A. Black, "Supramolecular Stereochemistry and Crystal Growth in Channel Inclusion Compounds," 18th International Conference on the Chemistry of the Organic Solid State, Merida, Venezuela, July 8-13, 2007. I also gave a lecture entitled, "Venezuela Birds - A Great National Heritage" as the last talk of the conference.

68. Invited Lecture: M. D. Hollingsworth, "Molecular Recognition in Channel Inclusion Compounds - Or Not!" School on Materials Applications of the Organic Solid State, Merida, Venezuela, July 2-7, 2007.

68. Invited Lecture: M. D. Hollingsworth, "Ferroelastic and Ferroelectric Materials - Properties and Design," School on Materials Applications of the Organic Solid State, Merida, Venezuela, July 2-7, 2007.

68. Invited Lecture: M. D. Hollingsworth, "The Role of Local and Long-range Stress in Solid State Photoreactions," School on Materials Applications of the Organic Solid State, Merida, Venezuela, July 2-7, 2007.

67. Invited Lecture: M. D. Hollingsworth, J. R. Rush, J. Bacsa, F. Guillaume, A. Desmedt, M. J. Abel, and A. A. Black, "Supramolecular Stereochemistry and Crystal Growth in Channel Inclusion Compounds," XIth International Seminar on Inclusion Compounds (ISIC–11), Kyiv, Ukraine, June 10 - 15, 2007.

66. Contributed Lecture: M. D. Hollingsworth, J. R. Rush, M. L. Peterson, M. J. Abel, A. A. Black, D. S. Kesselring, A. G. Butenhoff, M. Dudley, B. Raghothamachar, "Memory and Perfection in Domain Switching Processes," 11th International Meeting on Ferroelectricity, Iguassu Falls, Brazil, Sept. 5-9, 2005.

65. Invited Lecture: M. D. Hollingsworth, "Memory and Perfection in Domain Switching Processes," Summer School on Stereochemical Aspects of Novel Materials, International Center for Materials Research, UCSB, Santa Barbara, CA., August 14-26, 2005.

64. Invited Lecture: M. D. Hollingsworth, "Supramolecular Stereochemistry and Crystal Growth in Channel Inclusion Compounds," Summer School on Stereochemical Aspects of Novel Materials, International Center for Materials Research, UCSB, Santa Barbara, CA., August 14-26, 2005.

63. Contributed Lecture: M. D. Hollingsworth, J. R. Rush, M. L. Peterson, M. J. Abel, A. A. Black, D. S. Kesselring, A. G. Butenhoff, "Memory and Perfection in Domain Switching Processes," 17th International Conference on the Chemistry of the Organic Solid State, UCLA, Los Angeles, CA, July 24-29, 2005.

62. Invited Lecture: M. D. Hollingsworth, J. R. Rush, J. Bacsa, F. Guillaume, A. Desmedt, M. J. Abel, and A. A. Black, "Static versus Dynamic Surface Roughening in the Crystal Growth of Channel Inclusion Compounds," 2005 American Conference on Crystal Growth and Epitaxy, Big Sky, MT, July 2005

61. Invited Lecture: M. D. Hollingsworth, J. R. Rush, M. L. Peterson, M. J. Abel, A. A. Black, D. A. Kesselring, A. G. Butenhoff, "Crystal Engineering, Crystal Growth and Memory Effects in Ferroelastics and Ferroelectrics," Workshop on Designing Non-Traditional Materials Based on Geometrical Principles Hanover, Germany, June 20-22, 2005.

60. Invited Lecture: M. D. Hollingsworth, J. R. Rush, M. L. Peterson, M. Dudley, B. Ragothamachar, M, J. Abel, A. A. Black, "Memory, Perfection and Rubber-like Behavior in Ferroelastic and Ferroelectric Inclusion Compounds," American Crystallographic Association National Meeting, Orlando, FL, May 2005.

59. Contributed Lecture: M. D. Hollingsworth, M. L. Peterson, J. R. Rush, M. J. Abel, A. A. Black, D. A. Kesselring, A. G. Butenhoff, K. L. Pate, and J. A. Cooper, "From Ferroelastics to Ferroelectrics," Midwest Regional ACS Meeting, Manhattan, KS Oct. 2004.

58. Invited Lecture: M. D. Hollingsworth, M. L. Peterson, J. R. Rush, M. J. Abel, A. A. Black, D. A. Kesselring and A. G. Butenhoff, "From Ferroelastics to Ferroelectrics," 39th Congress of the Mexican Chemical Society, Merida, Mexico, Oct. 2004.

57. Contributed Lecture: M. D. Hollingsworth, J. Bacsa, C. F. Campana and M. L. Peterson, "Translational Disorder and Crystal Growth Mechanisms in Channel Inclusion Compounds," American Crystallographic Association National Meeting, Chicago, IL, July 2004

56. Plenary Lecture: M. D. Hollingsworth, J. R. Rush, M. L. Peterson, B. L Champion, M. J. Abel, and A. A. Black, "Symmetry Reduction in Ferroelastics: A General Approach to New Ferroelectric Materials?" Sixteenth International Conference on the Chemistry of the Organic Solid State, Sydney, Australia, July 13-18, 2003.

55. Poster: M. D. Hollingsworth, J. R. Rush, J. Bacsa, F. Guillaume, A. Desmedt, E. Elisabeth, M. J. Abel, and A. A. Black, "Static versus Dynamic Surface Roughening in the Crystal Growth of Channel Inclusion Compounds," Sixteenth International Conference on the Chemistry of the Organic Solid State, Sydney, Australia, July 13-18, 2003.

54. Invited Lecture: M. D. Hollingsworth, M. L. Peterson, M. J. Abel, A. A. Black, D. A. Higgins, G. Springer, J. C. Desper, J. Bacsa, M. Dudley, B. Ragothamachar, **"**Ferroelastic and Ferroelectric Domain Switching in Organic Inclusion Compounds," XIIth International Symposium on Supramolecular Chemistry, Oct. 6-11, 2002, Eilat, Israel.

53. Contributed Lecture: Hollingsworth, M. D., Peterson, M. L., Bacsa, J., Dudley, M., and Ragothamachar, B., "Twinning, Epitaxy, and Domain Switching in Ferroelastic Inclusion Compounds," NASA Microgravity Materials Science Conference, Huntsville, AL, June 25-27, 2002.

52. Invited Lecture: Hollingsworth, M. D., Peterson, M. L., Bacsa, J., Champion, B. L., Cooper, J. A. "New Tools for Studying Ferroelastic and Ferroelectric Domain Switching," American Conference on Crystal Growth/West, Lake Tahoe, CA, June 2002.

51. Invited Lecture: Hollingsworth, M. D., Peterson, M. L., Bacsa, J., Champion, B. L., "New Tools for Studying Ferroelastic and Ferroelectric Domain Switching," American Crystallographic Association National Meeting, San Antonio, TX, May 2002.

50. Contributed Lecture: Hollingsworth, M. D., Peterson, M. L., Kesselring, D. S., Butenhoff, A. G., Higgins, D. A., Springer, G., "A New Family of Ferroelastic and Ferroelectric Calixarenes," Fifteenth International Conference on the Chemistry of the Organic Solid State, Mainz, Germany, July 29 - August 3, 2001.

49. Poster: Pate, K. L., Peterson, M. L., Dinkelmeyer, B. D., Brown, M. E., Hollingsworth, M. D. "Failure of the topochemical postulate during a phase transition in a ferroelastic inclusion compound," poster presented at the Fifteenth International Conference on the Chemistry of the Organic Solid State, Mainz, Germany, July 29 - August 3, 2001.

48. Poster: Peterson, M. L., Hollingsworth, M. D., Dudley, M. and Ragothamachar, B. "Synchrotron white beam X-ray topography of domain switching in ferroelastic inclusion compounds," poster presented at the Fifteenth International Conference on the Chemistry of the Organic Solid State, Mainz, Germany, July 29 - August 3, 2001.

47. Invited Lecture: Hollingsworth, M. D.; Peterson, M. L., Geiger, T. A., Pate, K. L., Kesselring, D. S. and Butenhoff, A. G. "Ferroelastic and Ferroelectric Domain Switching in Organic Inclusion Compounds." Southwest-Southeast Regional ACS meeting, New Orleans, LA, Dec. 6-8, 2000.

46. Invited Lecture: Hollingsworth, M. D., Peterson. M. L., Dinkelmeyer, B., Kesselring, D. S., Butenhoff, A. G., "Ferroelastic and Ferroelectric Domain Switching in Organic Inclusion Compounds," Dynamics and Transformations of Molecular Materials (Eighth Polish-French Chemistry Seminar), Czocha Castle, Poland, Sept. 13-17, 2000.

45. Poster: Hollingsworth, M. D., Peterson, M. L., Dudley, M., Ragothamachar, B. "Synchrotron White Beam X-ray Topography Studies of Cooperativity and Impurity Control in Ferroelastic Domain Switching Processes." Dynamics and Transformations of Molecular Materials (Eighth Polish-French Chemistry Seminar), Czocha Castle, Poland, Sept. 13-17, 2000.

44. Invited Lecture: Hollingsworth, M. D., "Ferroelastic and Ferroelectric Domain Switching in Organic Inclusion Compounds," Gordon Conference on "Organic Structures and Properties: Extended Systems," Connecticut College, June 17-22, 2000.

43. Poster: Peterson, M. L., Hollingsworth, M. D., Brown, M. E., Dudley, M., Raghothamachar, B., "Tailor-made Impurity Control of Elastic Versus Plastic Domain Switching in Ferroelastic Inclusion Compounds," Gordon Conference on "Organic Structures and Properties: Extended Systems," Connecticut College, June 17-22, 2000.

42. Poster: Hollingsworth, M. D., Brown, M. E., Peterson, M. L., Dudley, M., Raghothamachar, B., "Tailor-made Impurity Control of Elastic Versus Plastic Domain Switching in Ferroelastic

Inclusion Compounds," NASA Microgravity Materials Science Conference, Huntsville, AL June 5-8, 2000.

41. Poster: Hollingsworth, M. D., Brown, M. E., Dudley, M., Chung, H., "Synchrotron White Beam X-ray Topography of Domain Switching and Twinning in Organic Inclusion Compounds," Minisymposium on Physical and Biophysical Chemistry, Kansas State University, Manhattan, KS, Oct. 30, 1999.

40. Poster: Hollingsworth, M. D.; Brown, M. E.; Werner-Zwanziger, U.; Chaney, J. D. "Spring-loaded Phase Transitions in Urea Inclusion Compounds," Fourteenth International Conference on the Chemistry of the Organic Solid State, Cambridge, U.K., July 25-30, 1999.

39. Contributed Lecture: Hollingsworth, M. D., Kesselring, D., Dinkelmeyer, B., Brown, M. E., "Crystal Growth, Domain Switching and the Search for Ferroelectric Behavior in Calixarene Solid Solutions," Fourteenth International Conference on the Chemistry of the Organic Solid State, Cambridge, U.K., July 25-30, 1999.

38. Invited Lecture: Hollingsworth, M. D., U. Werner-Zwanziger, J. D. Chaney, and M. E. Brown, "Domain Switching and Phase Transitions as Models for Solid State Reactions," US-Japan workshop on Solid State Chemistry, Lake Arrowhead, CA, Dec. 6-10, 1998.

37. Invited Lecture: Hollingsworth, M. D., J. D. Chaney, M. E. Brown, U. Werner-Zwanziger, "Domain and Phase Growth in Organic Inclusion Compounds," American Crystallographic Society, Annual Meeting, symposium on "Crystal Growth: Techniques and Mechanisms," July 24-28, 1998.

36. Invited Lecture: Hollingsworth, M. D. and co-workers, "Domain Switching as a Model for Solid State Reactions," 27th Reactions Mechanisms Conference, Asilomar Conference Center, Carmel, CA, June 28-July 3, 1998.

35. Plenary Lecture: Hollingsworth, M. D. "Cooperative Processes and Domain Switching in Organic Inclusion Compounds," Organic Crystal Chemistry Symposium (OCC97), Rydzyna, Poland, 17-21 August 1997.

34. Plenary Lecture: Hollingsworth, M. D., "Crystal Growth and Domain Switching in Organic Inclusion Compounds," Thirteenth International Conference on the Chemistry of the Organic Solid State, Stony Brook, NY, July 13-18, 1997.

33. Poster: U. Werner-Zwanziger, J.D. Chaney, M.E. Brown, E.J. Still, and M.D. Hollingsworth, "^{2}H NMR Studies of Guest-Induced Strain in Commensurate Inclusion Compounds of Urea," Thirteenth International Conference on the Chemistry of the Organic Solid State, Stony Brook, NY, July 13-18, 1997.

32. Poster: J. D. Chaney, H. Chung, M. E. Brown, M. D. Hollingsworth, and M. Dudley, "Synchrotron White Beam X-ray Topography and X-ray Crystallographic Studies of Domains

and Domain Switching in Channel Inclusion Compounds," Thirteenth International Conference on the Chemistry of the Organic Solid State, Stony Brook, NY, July 13-18, 1997.

31. Invited lecture: M. D. Hollingsworth and co-workers, "Design and Growth of Ferroelastic Inclusion Crystals" Gordon Conference on Physical Organic Chemistry, Holderness School, New Hampshire, June 1997.

30. Invited lecture: M. D. Hollingsworth and co-workers, "Ferroelastic Domain Switching as a Model for Solid State Reactivity," symposium on "Modification of Chemical Behavior by Use of Supramolecular Assemblies" at the 1997 Canadian Society for Chemistry National Meeting, Windsor, ON, June 1-5, 1997

29. Invited lecture: M. D. Hollingsworth, Michael E. Brown, Jason D. Chaney, Ulrike Werner-Zwanziger and Elizabeth A. Crane, "Topology, Architecture and Tailor-made Impurities in Ferroelastic Inclusion Compounds, symposium on "Molecular Design for Self-Assembled Structures: Principles and Applications," at the Central Regional ACS Meeting, Midland, MI, May 28-30, 1997.

28. Invited lecture: M. D. Hollingsworth, "Superstructure Control of Shape and Ferroelasticity of Urea Inclusion Compounds," 4th NSF-Sponsored Materials Chemistry Workshop, Philadelphia, PA, Oct. 17-20, 1996.

27. Invited lecture: M. D. Hollingsworth, M. E. Brown, U. Werner-Zwanziger, J. D. Chaney, E. A. Crane and E. J. Still, "Superstructure Control of Crystal Growth and Ferroelasticity in Urea Inclusion Compounds," Ninth International Symposium on Molecular Recognition and Inclusion, Lyon, France, Sept. 7-12, 1996

26. Poster: M. D. Hollingsworth, M. E. Brown, J. D. Chaney, U. Werner-Zwanziger, E. J. Still, J. A. Vanecko, "Cooperative Mechanisms in Ferroelastic Inclusion Compounds," 26th Reaction Mechanisms Conference, Stony Brook, NY, June 7-12, 1996.

25. Invited lecture: M. D. Hollingsworth, M. E. Brown, J. D. Chaney, U. Werner-Zwanziger, E. J. Still, J. A. Vanecko, "Superstructure Control of Shape and Elasticity of Urea Inclusion Compounds," NATO Advanced Research Workshop, Self-Assembly in Synthetic Chemistry, Val Morin, Quebec, May 16-21, 1996.

24. Invited lecture: M. D. Hollingsworth, U. Werner-Zwanziger and M. E. Brown, "Spectroscopic and Structural Studies of Functional Group Pairs in Channel Inclusion Compounds," International Congress of Pacific Basin Societies (PACIFICHEM '95), Honolulu, HI, Dec. 17-22, 1995 (Symposium on Role of Spectroscopic Methods in Modern Inorganic Chemistry).

23: Invited short talk: M. D. Hollingsworth, "Crystal Growth and Ferroelasticity in Organic Inclusion Compounds," NATO Advanced Research Workshop, Crystals: Supramolecular Materials," Sestri Levante, Italy, August 1995.

22. Poster: M. E. Brown and M. D. Hollingsworth, "The Role of Superstructures in the Crystal Growth and Ordering of Urea Inclusion Compounds," NATO Advanced Research Workshop, Crystals: Supramolecular Materials," Sestri Levante, Italy, August 1995.

21. Contributed lecture: M. D. Hollingsworth, M. E. Brown and A. D. Hillier, "The Role of Superstructures in the Crystal Growth and Ordering of Supramolecular Assemblies," Third International Workshop on Crystal Growth of Organic Materials, Washington, D. C. August 1995.

20. Poster: M. D. Hollingsworth and M. E. Brown, "The Role of Superstructures in the Crystal Growth and Ordering of Supramolecular Assemblies," 8th International Symposium on Molecular Recognition and Inclusion, Carleton University, Ottawa, Ontario, August 1994.

19. Poster: M. D. Hollingsworth, M. E. Brown, A. E. Aliev and K. D. M. Harris, "Crystallographic and Magnetic Resonance Anomalies in *n*-Alkanone/Urea Inclusion Compounds," Eleventh International Conference on the Chemistry of the Organic Solid State, Jerusalem, Israel, July 1993.

18. Poster: M. D. Hollingsworth, C. R. Goss and K. Folting, "Crystal Packing and Interactions in Primary N-Alkyl Formamides," Eleventh International Conference on the Chemistry of the Organic Solid State, Jerusalem, Israel, July 1993.

17. Plenary Lecture: Hollingsworth, M. D., Brown, M. E., John C. Huffman and O. H. Han, "Functional Group Recognition in Channel Inclusion Compounds," Eleventh International Conference on the Chemistry of the Organic Solid State, Jerusalem, Israel, July 1993.

16. Invited Lecture: Hollingsworth, M. D., "Functional Group Recognition in Channel Inclusion Compounds," Gordon Conference on Physical Organic Chemistry, June 1993, The Holderness School, NH.

15. Invited Lecture: Hollingsworth, M. D., Twelfth NSF Workshop on Reactive Intermediates, June 1993, Lake Tahoe, CA.

14. Contributed lecture: Hollingsworth, M. D., J. C. Huffman, S. P. Smart and K. D. M. Harris, "Functional Group Recognition and Non-recognition in Channel Inclusion Compounds," American Crystallographic Association National Meeting, Pittsburgh, PA, August 1992

13. Contributed Lecture: Hollingsworth, M. D., B. D. Santarsiero, J. C. Huffman, C. R. Goss, K. Folting, H. O. Mahamat and L. Di, "Packing Efficiency and Interactions in Channels and Layers," 4th Midwest Organic Solid State Chemistry Symposium, Lincoln, Nebraska, June 1992.

12. Poster: Hollingsworth, M. D., Goss, C. R, Harris, K. D. M., Santarsiero, B. D., "Long Range Dipole Organization and Crystal Habits of n-Alkanone/Urea Inclusion Compounds," Tenth International Conference on the Chemistry of the Organic Solid State, Vancouver, B.C., July 1991.

11. Poster: Hollingsworth, M. D., K. D. M. Harris, C. R. Goss, C. M. Bigam and A. R. Palmer, "Toward a Scale of Functional Group Interaction Energies: Equilibrium Control of Guest Orientations in Channel Inclusion Compounds of Perhydrotriphenylene," Sixth International Symposium on Molecular Recognition and Inclusion, Berlin, Federal Republic of Germany, Sept. 10-14, 1990.

10. Poster: M. D. Hollingsworth, N. Cyr, J. Huang, S. B. Rusch and C. J. Nichols, "Functional Group Recognition in Channel Inclusion Complex Formation," Ninth International Conference on the Chemistry of the Organic Solid State, Como, Italy, July 2-7, 1989.

9. Invited keynote lecture: M. D. Hollingsworth, "Cooperativity and Molecular Recognition in Crystals," Second Annual Midwest Organic Solid State Chemistry Symposium, Minneapolis, MN, June 10-11, 1989.

8. Invited Lecture: M. D. Hollingsworth, J. Huang, K.D.M. Harris and N. Cyr, "Studying Substituent Effects in Channels," 72nd Canadian Chemical Conference and Exhibition, Victoria, B.C., June 4-8, 1989.

7. Awards Address: M. D. Hollingsworth, "Infrared Studies of CO_2 Dimers as a Probe of Local Stress in Solid State Peroxide Reactions," for receipt of the Distinguished Dissertation Award (Physical Sciences and Engineering, 1982-1986), Annual Meeting of the Northeastern Association of Graduate Schools, State College, PA, April 1988.

6. Plenary Lecture: M. D. Hollingsworth, "Radical Reactions in Crystalline Hosts," Eighth International Congress on the Chemistry of the Organic Solid State, Lyon-Villeurbanne, France, July 1987.

5. Awards address: Hollingsworth, M. D. and J. M. McBride, "Local Stress and Mechanism in Solid State Photochemistry," for receipt of the 1987 Nobel Laureate Signature Award for Graduate Education in Chemistry, 193rd ACS National Meeting, Denver, Colo., April 1987

4. Poster: Hollingsworth, M. D. and J. M. McBride, "Infrared Studies of Long-Range Stress in Solid-State Peroxide Photoreactions," The Eighth International Congress on the Chemistry of the Organic Solid State, Lyon-Villeurbanne, France, July 1987.

3. Poster: Hollingsworth, M. D. and J. M. McBride, "Infrared Studies of Photoreactions within Crystalline Acetyl Benzoyl Peroxide," The Eighth International Congress on the Chemistry of the Organic Solid State, Lyon-Villeurbanne, France, July 1987.

2. Poster: Harris, K. D. M., M. D. Hollingsworth, W. Jones and J. M. Thomas, "Structure and Reactivity of Diacyl Peroxide/Urea Inclusion Complexes," The Eighth International Congress on the Chemistry of the Organic Solid State, Lyon-Villeurbanne, France, July 1987.

1. Poster: Harris, K. D. M., M. D. Hollingsworth, W. Jones, J. M. Thomas and W.-N. Wang, "A Comparison of Photoreactivity of Diacyl Peroxides in Urea and Aluminosilicate Hosts," 4th International Symposium on Inclusion Phenomena, Lancaster, U. K. July 1986.

CO-AUTHORED CONTRIBUTIONS TO CONFERENCES BY STUDENTS AND COLLABORATORS

52. Poster: I. Frantsuzov, C. Mariette, Bo Wang, L. Guerin, P. Rabiller, B. Toudic, and Mark D. Hollingsworth, "Frustrated Phases in Higher Dimensions," British Crystallographic Association Spring Meeting, Nottingham, U.K., April 15-18, 2019.

51. Contributed talk: Ilya Frantsuzov, Bo Wang, Mark Hollingsworth, Shane M. Nichols, Philippe Rabiller, Céline Mariette, Laurent Guérin, Bertrand Toudic, "Self-Compression in Urea Inclusion Compounds," Aperiodic 2018, Ames, IA, July 8-13, 2018.

50. Contributed talk: Adam Lechner, Bo Wang, Mark D. Hollingsworth, "Polymorphism in Fumaronitrile and Growth of Cocrystals with Urea," 27th Midwest Organic Solid State Chemistry Symposium, Kansas State University, Manhattan, KS June, 2017.

49. Contributed talk: Bo Wang, Ilya Frantsuzov, and Mark D. Hollingsworth, "Self-compression in phase transitions of a urea inclusion compound," 27th Midwest Organic Solid State Chemistry Symposium, Kansas State University, Manhattan, KS June, 2017.

48. Contributed talk: Bo Wang, Ilya Frantsuzov, and Mark D. Hollingsworth, "Crystal Structure, Phase Transitions, and Self-compression of a Urea Inclusion Compound," 25th Midwest Organic Solid State Chemistry Symposium, West Lafayette, IN, June, 2015.

47. Contributed talk: Ilya Frantsuzov, Bo Wang, Keith E. Alquist III, Roman B. Gajda, Kevin L. Pate, Angela D. Adams, Fharhod Nozirov, Bo Wang, and Mark D. Hollingsworth, "The Missing Phase Transition in 1,6-Dicyanohexane/Urea," 25th Midwest Organic Solid State Chemistry Symposium, West Lafayette, IN, June, 2015.

46. Contributed talk: B. Wang and M. D. Hollingsworth,"Self-compression in Urea Inclusion Compounds," 24th Midwest Organic Solid State Chemistry Symposium, Iowa City, IA, June, 2014.

45. Poster: S. M. Nichols, Bo Wang, Chunhua Hu, Mark Hollingsworth, "Crystal Structure of a Six-Fold Non-Merohedrally Twinned Inclusion Compound," Bruker-AXS/MIT Symposium 2014, Feb. 21-23, 2014, Cambridge, MA. Shane Nichols won the poster prize for his poster at this meeting.

44. Contributed talk: B. Wang, M. D. Hollingsworth, S. M. Nichols, J. Bacsa, X. Li, "Crystal Growth and Surface Roughening in Commensurate and Incommensurate Channel Inclusion Compounds," 2013 Midwest Regional Meeting of the American Chemical Society, Springfield, MO, Oct. 16-18, 2013.

43. Contributed talk: B. Wang, M. L. Peterson, and M. D. Hollingsworth, "Cooperative Guest-Host-Guest Recognition in Ferroelastic and Ferroelectric Calixarenes," 23rd Midwest Organic Solid State Chemistry Symposium, Lexington, KY, June, 2013

42. Invited talk: M. L. Peterson, K. L. Pate, B. D. Dinkelmeyer, K. E. Alquist III, M. D. Hollingsworth, "Space Group Assignment and Evaluation of End-for-End Guest Disorder in $Cl(CH_2)_6CN$/Urea," Transactions Symposium, American Crystallographic Association National Meeting, Boston, MA, July 2012.

41. Poster: B. Wang, M. L. Peterson, S. M. Nichols, E. J. Chan, and M. D. Hollingsworth, "Cooperative Guest-Host-Guest Recognition in Ferroelastic and Ferroelectric Calixarenes," American Crystallographic Association National Meeting, Boston, MA, July 2012. Bo Wang won the CrystEngComm Poster Prize for this poster.

40. Contributed Talk: B. Wang, M. D. Hollingsworth, E. Kolodziejczyk, J. Bacsa, "Screw-like Twinning and Surface Roughening in the Growth of Channel Inclusion Compounds," 22nd Midwest Organic Solid State Chemistry Symposium, Springfield, MO, June 2012.

39. Poster: E. J. Chan, M. D. Hollingsworth, "Single Crystal X-ray Diffuse Scattering from Supra-molecular Assemblies," XXII Congress and General Assembly of the International Union of Crystallography, Madrid, Spain, August 22-30, 2011.

38. Contributed talk: Céline Mariette, Philippe Rabiller, Bertrand Toudic, Laurent Guérin, Mark Hollingsworth, "One dimensional confinement in aperiodic materials. International school and symposium on multifunctional molecule-based materials," 2011, Argonne National Laboratory, United States.

37. Poster: P. Rabiller, L. Guérin, C. Mariette, B. Toudic, C. Ecolivet, M. Hollingsworth, "Huge period *versus* aperiodicity in organic host guest systems," XXII Congress and General Assembly of the International Union of Crystallography, Madrid, Spain, August 22-30, 2011.

36. Contributed talk: L. Guérin, C. Mariette, B. Toudic, P. Rabiller, C. Ecolivet, M. Hollingsworth, "Diffuse Scattering in One Dimensional 'Liquid-like' Aperiodic Composites," XXII Congress and General Assembly of the International Union of Crystallography, Madrid, Spain, August 22-30, 2011.

35. Contributed talk: A. D. Adams, J. Abeykoon, R. Gajda, B. Wang, S. M. Nichols, M. D. Hollingsworth, "Correlating Optical Rotation with Absolute Configuration of Urea Inclusion Compounds," 21st Midwest Organic Solid State Chemistry Symposium, Charleston, IL June 2011.

34. Contributed talk: Shane M. Nichols, E. J. Chan, B. Wang, M. D. Hollingsworth, "Synchrotron Studies of Stressed Crystals," 21st Midwest Organic Solid State Chemistry Symposium, Charleston, IL June 2011.

33. Poster: M. Huard, B. Toudic, C. Ecolivet, P. Rabiller, C. Odin, P. Bourges, M. D. Hollingsworth, T. Brewczewski, "Instabilités Structurales de Cristaux Moléculaires Apériodiques Hôte-invite," X-Ray and Materials 2009, Paris, France, Dec. 11, 2009.

32. Poster: M. Huard, B. Toudic, C. Ecolivet, P. Rabiller, C. Odin, P. Bourges, M. D.

Hollingsworth, T. Brewczewski, "Structural Instabilities in Aperiodic Host-guest Crystals," 5th International Symposium on Molecular Materials: Electronics, Photonics and Spintronics, Rennes, France, Oct. 2009

31. Poster: M. Huard, B. Toudic, C. Ecolivet, P. Rabiller, C. Odin, P. Bourges, M. D. Hollingsworth, T. Brewczewski, "Brisures de Symétrie dans un Superspace Cristallographique," Contribution of Symmetries in Condensed Matter School, Giens, France, May, 2009.

30. Poster: B. Toudic, M. Huard, C. Ecolivet, P. Rabiller, C. Odin, P. Bourges, M. D. Hollingsworth, T. Brewczewski, "Étude de systèmes moléculaires apériodiques," Colloque de l'Association Française de Cristallographie, Rennes, France, July 7-10, 2008.

29. Poster: B. Toudic, P. Rabiller, C. Odin, L. Bourgeois, C. Ecolivet, P. Garcia, F. Le Gac, P. Bourges, F. Guillaume, T. Brewczewski, M.D. Hollingsworth, "New Crystallographic Features in Aperiodic Supramolecular Crystals," XX Congress of the International Union of Crystallography, Florence, Italy, August 23-31, 2005.

28. Contributed Talk: J. R. Rush, M. D. Hollingsworth and M. J. Abel, "Polar Ordering and Electric Field-Induced Domain Reorientation in Ferroelastic Channel Inclusion Compounds," Midwest Regional ACS Meeting, Manhattan, KS Oct. 2004.

27. Contributed Talk: J. R. Rush, M. D. Hollingsworth and M. J. Abel, "Polar Ordering and Electric Field-Induced Domain Reorientation in Ferroelastic Channel Inclusion Compounds," American Crystallographic Association National Meeting, Chicago, IL, July 2004.

26. Contributed Talk: J. R. Rush, M. D. Hollingsworth and M. J. Abel, "Polar Ordering and Electric Field-Induced Domain Reorientation in Channel Inclusion Compounds," Fifteenth Midwest Organic Solid State Chemistry Symposium, Carbondale, IL June 2004.

25. Contributed Talk: J. R. Rush, M. J. Abel, A. A. Black and M. D. Hollingsworth, "Symmetry Reduction and Ordering in Urea Inclusion Compounds," Fourteenth Midwest Organic Solid State Chemistry Symposium, Minneapolis, MN, June 2003.

24. Contributed Talk: Abel, M. J., Black, A. A., Hollingsworth, M. D., "Factors Affecting Domain Reorientation in Ferroelastic Inclusion Compounds," Fourteenth Midwest Organic Solid State Chemistry Symposium, Minneapolis, MN, June 2003.

23. Contributed Talk: Pate, K. L., Hollingsworth, M. D., Peterson, M. L., Dinkelmeyer, B. D., Brown, M. E. "Nontopochemical motion during a phase transition in a ferroelastic inclusion compound," Twelfth Midwest Organic Solid State Chemistry Symposium, Lincoln, NE, June 2001.

22. Contributed Talk: Peterson, M. L., Hollingsworth, M. D., Brown, M. E., Butenhoff, A. G., "Ferroelectric 4-tert-Butylcalix[4]arene Inclusion Crystals," Twelfth Midwest Organic Solid State Chemistry Symposium, Lincoln, NE, June 2001.

21. Contributed Talk: Dinkelmeyer, B., Hollingsworth, M. D., Brown, M. E., 'Mechanism and Control of Ferroelastic Phase Transitions in Urea Inclusion Compounds Containing α,ω-Disubstituted Hexanes," Eleventh Midwest Organic Solid State Chemistry Symposium, West Lafayette, IN, June 9-10, 2000.

20. Contributed Talk: Peterson, M. L., Hollingsworth, M. D., Brown, M. E., Dudley, M., Raghothamachar, B., Dhanaraj, G., "Tailor-made Impurity Control of Elastic Versus Plastic Domain Switching in Ferroelastic Inclusion Compounds," Eleventh Midwest Organic Solid State Chemistry Symposium, West Lafayette, IN, June 9-10, 2000.

19. Contributed talk: M. E. Brown, U. Werner-Zwanziger, J. D. Chaney, E. J. Still, M. D. Hollingsworth, "Spring-loaded Phase Transitions in Channel Inclusion Compounds of Urea," Tenth Midwest Organic Solid State Chemistry Symposium, Indianapolis, IN, June 4-5, 1999.

18. Poster: U. Werner-Zwanziger, M. E. Brown, J. D. Chaney, E. J. Still, and M. D. Hollingsworth, "Guest Motions in Dihalohexane/Urea Inclusion Compounds studied by ^{2}H NMR," 29th AMPERE - 13th International Conference on Magnetic Resonance and Related Phenomena, Berlin, Germany, August 2-7, 1998.

17. Contributed lecture: M. E. Brown, J. D. Chaney, M. D. Hollingsworth, "Hydrogen-bonded Urea Inclusion Compounds and their Ferroelastic Phase Transitions," Ninth Midwest Organic Solid State Chemistry Symposium, Kansas State University, Manhattan, KS, June 12-13, 1998.

16. Poster: U. Werner-Zwanziger, M. E. Brown, J. D. Chaney, E. J. Still, and M. D. Hollingsworth, "Guest Motions in Dihalohexane/Urea Inclusion Compounds studied by ^{2}H NMR," 39th Experimental Nuclear Magnetic Resonance Conference, Pacific Grove, CA March 22-27, 1998.

15. Poster: U. Werner-Zwanziger, M. E. Brown, J. D. Chaney, E. J. Still, and M. D. Hollingsworth, "^{2}H NMR Studies of Dihalohexane/Urea Inclusion Compounds," Gordon Research Conference on Magnetic Resonance, Henniker, NH, June 22-27, 1997.

14. Poster: U. Werner-Zwanziger, M. E. Brown, J. D. Chaney, E. J. Still, and M. D. Hollingsworth, "^{2}H NMR Studies of Commensurate Dihalohexane/Urea Inclusion Compounds," 38th Experimental Nuclear Magnetic Resonance Conference, Orlando, Florida, March 23-27, 1997.

13. Contributed lecture: U. Werner-Zwanziger, M. E. Brown, J. D. Chaney, E. J. Still, J. A. Vanecko and M. D. Hollingsworth, "Deuterium NMR Studies of Commensurate Urea Inclusion Compounds," Chicago Area NMR Discussion Group, Nov. 2, 1996.

12. Contributed lecture: M. E. Brown, J. D. Chaney, E. J. Still, E. A. Crane, J. A. Vanecko and M. D. Hollingsworth, "Designing Urea Inclusion Compounds," Eighth Midwest Organic Solid State Chemistry Symposium, Lincoln, NE, June 1996.

11. Contributed lecture: U. Werner-Zwanziger, J. D. Chaney, E. J. Still, E. A. Crane, J. A. Vanecko and M. D. Hollingsworth, "NMR Studies of Urea Inclusion Compounds," Eighth Midwest Organic Solid State Chemistry Symposium, Lincoln, NE, June 1996.

10. Poster: H. Chung, M. Dudley, M. E. Brown and M. D. Hollingsworth, "Synchrotron White Beam X-ray Topography Characterization of Defect Structures in 2,10-Undecanedione/urea Inclusion Compounds," Twelfth International Conference on the Chemistry of the Organic Solid State, Matsuyama, Japan, July 9-14, 1995 (Chung and Dudley in attendance).

9. Contributed lecture: M. E. Brown and M. D. Hollingsworth, "Ferroelastic Inclusion Compounds," Seventh Midwest Organic Solid State Chemistry Symposium, Bloomington, IN, June, 1995

8. Contributed lecture: J. D. Chaney, M. D. Hollingsworth, K. Folting and C. R. Goss, "C-H---O Hydrogen Bonding at Work: Crystal Packing Patterns of Even-Chain bis-Formamides," Seventh Midwest Organic Solid State Chemistry Symposium, Bloomington, IN, June 1995.

7. Contributed lecture: U. Werner-Zwanziger, M. E. Brown and M. D. Hollingsworth, "Incommensurate Inclusion Compounds: Ideal Hosts for Studies of Functional Group Interactions," Seventh Midwest Organic Solid State Chemistry Symposium, Bloomington, IN, June 1995.

6. Paper: U. Werner-Zwanziger, M. E. Brown and M. D. Hollingsworth, "NMR Studies of Functional Group Interactions in Incommensurate Channel Inclusion Compounds," 1994 Chicago Area NMR Discussion Group, Nov. 12, 1994.

5. Poster: U. Werner-Zwanziger, M. E. Brown and M. D. Hollingsworth, "Incommensurate Inclusion Compounds: Ideal Systems for Studies of Functional Group Interactions," XXVII Congress Ampere, Kazan, Russian Federation, Aug. 21, 1994.

4. Poster: U. Werner-Zwanziger, M. E. Brown and M. D. Hollingsworth, "Incommensurate Inclusion Compounds: Ideal Systems for Studies of Functional Group Interactions," Gordon Research Conference of Order/Disorder in Solids, New London, New Hampshire, Aug. 7, 1994.

3. Contributed Lecture: M. E. Brown and M. D. Hollingsworth, "The Role of Superstructures in the Ordering and Crystal Growth of Supramolecular Assemblies" 6th Midwest Solid State Organic Chemistry Symposium, Minneapolis, MN, June 11, 1994.

2. Contributed Lecture: M. D. Hollingsworth, M. E. Brown, A. E. Aliev and K. D. M. Harris, "The 2-Undecanone/Urea Conundrum," Fifth Midwest Organic Solid State Chemistry Conference, Purdue University, West Lafayette, IN, June 1993.

1. Poster: Harris, K. D. M., S. P. Smart and M. D. Hollingsworth, "Interchannel Ordering of Guest Molecules in Urea Inclusion Compounds," International Union of Crystallography Congress, Bordeaux, France, July 1990.

OTHER SEMINARS AND INVITED CHEMISTRY LECTURES

2018
Institute of Physics, University of Rennes, Rennes, France

2015
Chemistry Department, Whitman College

2014
Chemistry Department, Emory University

2013
Chemistry Department, Syracuse University

2011
Chemistry Department, Truman State University (invited by the student ACS affiliate chapter)

2009
Chemistry Department, Johns Hopkins University
Physics Department, Johns Hopkins University

2006
Chemistry Department, University of Washington
Groupe Matière Condensée et Matériaux, University of Rennes, Rennes, France
Laboratoire de Physico-Chimie Moléculaire, University of Bordeaux
Laboratoire de Chimie de Coordination Organique, Université Louis Pasteur, Strasbourg
Laboratory for Chemical and Mineralogical Crystallography, University of Bern

2005
Chemistry Department, Truman State University (invited by the student ACS affiliate chapter)
Department of Physical Chemistry, University of Cambridge
Groupe Matière Condensée et Matériaux, University of Rennes, Rennes, France
Laboratoire de Physico-Chimie Moléculaire, University of Bordeaux
Chemistry Department, University of Cardiff
TransForm Pharmaceuticals, Lexington, MA

2003
Chemistry Department, University of New Orleans, New Orleans, LA
Chemistry Department, Tulane University, New Orleans, LA

2002
Chemistry Department, Cornell College, Mt. Vernon, IA
Chemistry Department, Central Missouri State University, Warrensburg, MO
Chemistry Dept., Emporia State University, Emporia, KS
Chemistry Dept., University of Iowa, Iowa City, IA
Chemistry Department, Kansas State University (physical chemistry colloquium)

Chemistry Department, Kansas State University (physical chemistry colloquium)
Chemistry Undergrad. Journal Club, Kansas State University, "CFCs and Stratospheric Ozone Depletion"

2001
Chemistry Department, Lamar University, Beaumont, Texas
TransForm Pharmaceuticals, Lexington, MA
Chemistry Department, College of William and Mary, Williamsburg, VA
Chemistry Department, Grinnell College, Grinnell, IA
Chemistry Department, Drake University, Des Moines, IA
Chemistry Department, Trinity University, San Antonio, Texas
Groupe Matière Condensée et Matériaux, University of Rennes, Rennes, France
Chemistry Department, University of Nebraska at Lincoln
Chemistry Department, Georgetown University, Washington, D. C.
Chemistry Department, Kansas State University (physical chemistry colloquium)

2000
Chemistry Department, Northern Arizona University
Chemistry Department, University of Missouri at Columbia

1998
Chemistry Department, University of Nebraska
Chemistry Department, Wesleyan University
Chemistry Department, Washington State University, Pullman, WA
Chemistry Department, Kansas State University

1997
Chemistry Department, University of Massachusetts at Amherst
Chemistry Department, Carnegie Mellon University
Chemistry Department, University of Utah
Chemistry Department, University of Colorado at Boulder
Chemistry Department, Pennsylvania State University
Chemistry Department, University of Wisconsin at Madison
Chemistry Department, Ohio State University
Chemistry Department, University of Minnesota

1996
Chemistry Department, University of Illinois at Urbana-Champaign

1995
Chemistry Department, Yale University, New Haven, CT
Chemistry Department, SUNY Stony Brook, Stony Brook, NY

1994
Chemistry Department, DePauw University, Greencastle, IN (recruiting talk)
Chemistry Department, Indiana University, Solid State Chemistry Seminar
Chemistry Department, Indiana University, Solid State Chemistry Seminar

1993
Chemistry Department, Carleton College, Northfield, Minn.
Chemistry Department, Indiana University, Solid-State Chemistry

1992
Chemistry Department, Indiana State University, Terre Haute, IN
Chemistry Department, Indiana University-Purdue University at Indianapolis
Chemistry Department, Purdue University, Materials Chemistry Series
Chemistry Department, Indiana University, Solid-State Chemistry

1991
Chemistry Department, Indiana University
Chemistry Department, University of Illinois
Chemistry Department, University of British Columbia

1990
Chemistry Department, University of Colorado at Boulder
Chemistry Department, Colorado State University
Chemistry Department, University of St. Andrews
Chemistry Department, Princeton University

1988
Chemistry Department, University of Alberta

1987
Chemistry Department, University of Calif. at Berkeley
Chemistry Department, University of Chicago
Chemistry Department, University of Calif. at Irvine
Chemistry Department, University of Calif. at Santa Barbara
Chemistry Department, University of Oregon
Chemistry Department, Yale University
Chemistry Department, University of Wisconsin
Chemistry Department, University of Alberta

1986
Chemistry Department, Brown University
Department of Physical Chemistry, University of Cambridge (two)
CICE Institute, Barcelona, Spain
The Royal Institution of Great Britain

GRANTS AND AWARDS FOR RESEARCH

Investigator, dates	Agency, Type, Grant Title	Amount
M. Hollingsworth December 2019	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions and Superspace	0.7 days of beam time

	Issues in Perfectly Oriented Crystals" (GUP-68774)	
M. Hollingsworth November 2019	Advanced Photon Source, Argonne National Laboratory, "Crystallography of challenging Host-guest systems" (GUP-66467)	0.7 days of beam time
M. Hollingsworth November 2019	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions and Superspace Issues in Organic Inclusion Compounds," (GUP-67310)	2 days of beam time
M. Hollingsworth September 2019	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions and Self-compression in Channel Inclusion Compounds," (GUP-66962)	1 day of beam time
M. Hollingsworth January 2019	Stanford Synchrotron Radiation Laboratory, "Synchrotron studies of phase transitions and structures of organic inclusion compounds," (SSRL-5323)	recommended - amount of beam time to be specified
M. Hollingsworth August 2016	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions and 'self-Compression' in aperiodic inclusion compounds" (GUP-42000) (12 days for life of proposal)	2 days of beam time (+ 3 days discretionary time)
M. Hollingsworth July 2015	Advanced Light Source, Lawrence Berkeley Laboratories, Structural Biology Rapid Access "Lock-in/Self-compression versus Incommensurate Modulation in Channel Inclusion Compounds." (SB-00439)	1.7 days of beam time
M. Hollingsworth March 2015	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions and 'self-Compression' in aperiodic inclusion compounds"	4 days of beam time granted
M. Hollingsworth March 2015	Advanced Light Source, Lawrence Berkeley Laboratories, Structural Biology Rapid Access "Lock-in phase transitions of channel inclusion compounds" (SB-00352)	1 day of beam time
M. Hollingsworth Jan. 2015	Advanced Light Source, Lawrence Berkeley Laboratories, Structural Biology Rapid Access "X-ray Studies of Self-compression	16 hours of beam time

	in Channel Inclusion Compounds"	
M. Hollingsworth Jan. 2015	Advanced Light Source, Lawrence Berkeley Laboratories, "Domain switching, phase transitions, and crystal growth in channel inclusion compounds" (ALS-06228)	2.3 days of beam time
M. Hollingsworth Dec. 2014	Advanced Light Source, Lawrence Berkeley Laboratories, "X-ray Studies of Self-compression in Channel Inclusion Compounds" Structural Biology Rapid Access (SB-00302)	1 day of beam time
M. Hollingsworth Nov. 2014	Advanced Light Source, Lawrence Berkeley Laboratories, Structural Biology Rapid Access	1.3 days of beam time
M. Hollingsworth Nov. 2014	Advanced Light Source, Lawrence Berkeley Laboratories, "Domain switching, phase transitions, and crystal growth in channel inclusion compounds" (ALS-06228)	2 days of beam time
M. Hollingsworth June 2014	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions, Domain Switching, and Crystal Growth in Periodic and Aperiodic Inclusion Compounds" (GUP-33163)	3 days of beam time
M. Hollingsworth April 2014	Laboratoire Léon Brillouin, "High-pressure neutron diffraction of a self-compressing inclusion compound" (LLB-12036)	one week of beam time
M. Hollingsworth March 2014	Diamond Light Source, Oxford, U.K., "High-resolution Studies of Organic Inclusion Compounds" Rapid-access proposal (MX5019)	1 day of beam time
M. Hollingsworth October 2013	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions, Domain Switching, and Crystal Growth in Periodic and Aperiodic Inclusion Compounds" (GUP-33163)	3 days of beam time
M. Hollingsworth October 2013	Advanced Light Source, Lawrence Berkeley Laboratories, "Self-compression" in Channel Inclusion Compounds" Rapid-access proposal (SB-00145)	2 days of beam time
M. Hollingsworth	Advanced Photon Source, Argonne National	3 days of

July 2013	Laboratory, "Phase Transitions, Domain Switching, and Crystal Growth in Periodic and Aperiodic Inclusion Compounds" (GUP-33163)	beam time
M. Hollingsworth March 2013	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions, Domain Switching, and Crystal Growth in Periodic and Aperiodic Inclusion Compounds" (GUP-33163)	3 days of beam time (12 days for life of proposal)
M. Hollingsworth Jan. 2012 - Dec. 2013	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions and Crystal Growth in Periodic and Aperiodic Inclusion Compounds" (GUP-28180)	1 day of beam time
M. Hollingsworth June 2012	Advanced Photon Source, Argonne National Laboratory, "Phase Transitions, Domain Switching, and Crystal Growth in Periodic and Aperiodic Inclusion Compounds" (GUP-30451)	10 days of beam time
M. Hollingsworth March 2012 (rapid access)	Advanced Photon Source, Argonne National Laboratory, "High-resolution X-ray Studies of Superstructures and Superspaces in Channel Inclusion Compounds" (GUP-29673)	3 days of of beam time
M. Hollingsworth Nov. 2011 - May, 2013	Stanford Synchrotron Radiation Laboratory, "Mechanistic studies of phase transitions and crystal growth in periodic and aperiodic composite crystals" (SSRL-3688)	recommended - amount of beam time to be specified
M. Hollingsworth Jan. 2011 – July 2012	Advanced Light Source, Lawrence Berkeley Laboratories, "Interfacial control of memory effects in ferroelastic inclusion compounds" (ALS-04767)	1.7 days of beam time
M. Hollingsworth Jan. 2011 - July 2012	Advanced Photon Source, Argonne National Laboratory, "Single Crystal Diffraction of Ferroelastic and Ferroelectric Inclusion Compounds" (GUP-22632)	3 days of beam time
M. Hollingsworth Oct. 2010 - Apr. 2012	Advanced Photon Source, Argonne National Laboratory, "Single Crystal Diffraction of Ferroelastic and Ferroelectric Inclusion Compounds" (GUP-21666)	3 days of beam time

M. Hollingsworth Jan. - June, 2011	Oak Ridge National Laboratory, "Absolute Configuration of Urea Inclusion Compounds from Neutron Diffraction of Stereospecifically Deuterated Crystals" (IPTS-3784)	4 days of beam time
M. Hollingsworth Jan. - June, 2011	Stanford Synchrotron Radiation Laboratory, "Interfacial Control of Memory Effects in Ferroelastic Inclusion Compounds" (SSRL-3354)	2 days of beam time
M. Hollingsworth Jan. - June, 2011	Advanced Light Source, Lawrence Berkeley Laboratories, "Single Crystal Diffraction of a Ferroelectric Calixarene Complex" (ALS-04106)	4 days of beam time
M. Hollingsworth Jan. - June, 2011	Advanced Light Source, Lawrence Berkeley Laboratories, "Single Crystal X-ray Diffraction of an Incommensurately Modulated Phase in an Aperiodic Inclusion Compound" (ALS-04103)	2.5 days of beam time
B. Toudic, P. Rabiller, M. D. Hollingsworth October 2008	SOLEIL Synchrotron, CRYSTAL Beamline, "Superspace Symmetry Breaking in Crystals of Tetradecane/urea"	2 days of beam time
M. D. Hollingsworth 7-1-2008 to 6-30-2011	National Science Foundation (Division of Chemistry), "Synthesis and Mechanistic Studies of New Series of Ferroelastic and Ferroelectric Crystals"	$390,000 + $78,000 equip. + travel supplements
M. D. Hollingsworth 1-1-2006 to 8-31-2008	American Chemical Society (PRF, Type AC "Crystal Growth, Polar Ordering and Domain Switching in Ferroelastoelectric Inclusion Compounds"	$80,000
M.D. Hollingsworth 4-1-00 to 11-30-04	National Aeronautics and Space Administration (Microgravity Materials Science Initiative - Research and Flight Experiment Opportunities), "Crystal Growth of New Families of Ferroelastic Materials"	$310,000
M. D. Hollingsworth Jan.-June, 2003	Department of Energy (Intense Pulsed Neutron Source, Argonne National Laboratories), "Neutron Diffraction of Formyl C-H---O Interactions"	~10 days of beam time
M. D. Hollingsworth Jan.-June, 2000	Department of Energy (Intense Pulsed Neutron Source, Argonne National Laboratories),	~10 days of beam time

	"Neutron Diffraction of Ferroelectric and Ferroelastic Calixarene Crystals"	
F. Guillaume M. D. Hollingsworth B. Toudic, C. Odin June, 2002	Berlin Experimental Neutron Scattering Center, "Molecular Dynamics in the Incommensurate 2-Decanone/urea crystal"	7 days of beam time
F. Guillaume M. D. Hollingsworth B. Toudic, C. Odin August 13-18, 2001	Berlin Experimental Neutron Scattering Center, "Molecular Dynamics in the Incommensurate 2-Decanone/urea crystal"	6 days of beam time
M. D. Hollingsworth, M. M. Collinson, D. A. Higgins, K. J. Klabunde, P. M. A. Sherwood 8-1-00 to 7-31-01	National Science Foundation, Division of Materials Research, "Acquisition of a Scanning Probe Microscope for Materials Research and Education,"	$100,000
M. D. Hollingsworth 4-1-97 to 3-31-01	National Science Foundation (Division of Materials Research), "Cooperative Phenomena and Domain Switching Processes in Organic Inclusion Compounds"	$243,500
M. D. Hollingsworth 6-1-95 to 5-31-00	National Science Foundation (Division of Materials Research), "Energetic and Structural Studies of Functional Group Pairs for Materials Research"	$300,000
M. D. Hollingsworth 4-23-96 to 4-22-97	Research Corporation, "Stereomicroscope for Studies of Organic Crystals and Inclusion Compounds"	$15,000 (+$7,500 match from IU)
M. D. Hollingsworth 7-1-95 to 8-31-97	American Chemical Society (Petroleum Research Fund, Type AC), "Crystal Engineering and Ordering in One, Two and Three Dimensions"	$50,000
M. D. Hollingsworth 7-1-95 to 5-31-96	National Science Foundation (Chemistry Division), "Equipment Supplement for CHE-9423726: Solid State NMR Console"	$37,450 (+$26,000 match from IU)
M. D. Hollingsworth 1993-1995	Cambridge Isotope Laboratories, "^{13}C Labeled Compounds for Spectroscopic Studies of Functional Group Interactions"	$3,000 in labeled compounds
M. D. Hollingsworth	Indiana University, Summer Faculty Fellowship	$4,500

Summer, 1992	"Structural Studies of Functional Group Pairs in Channel Inclusion Compounds"	
M. D. Hollingsworth 9-15-91 to 9-14-93	Alfred P. Sloan Foundation - Sloan Research Fellowship	$30,000
M. D. Hollingsworth 9-1-91 to 8-31-93	Petroleum Research Fund (Type AC) "A Scale of Functional Group Interaction Energies"	$40,000
M. D. Hollingsworth 1991-1993 (terminated 9-91)	NSERC, Operating, "Functional Group Interactions in Organic Crystals and Inclusion Compounds"	CAN$24,000
M. D. Hollingsworth 1989-1991	NSERC, Operating, "Inclusion Phenomena, Molecular Recognition and Intermolecular Forces"	CAN $46,980
M. D. Hollingsworth 1990	Univ. of Alberta, Central Research Fund Operating, "Solid-state NMR Studies in Colorado and Scotland"	CAN $1,600
M. D. Hollingsworth 1989	Univ. of Alberta, Central Research Fund, Operating, "Solid-state NMR Studies"	CAN $2,000
M. D. Hollingsworth 1988	Univ. of Alberta, Central Research Fund, Operating, "Spectroscopic and Mechanistic Studies of Reactions in Organic Solids"	CAN $4,000
M. D. Hollingsworth 1986-1987	NSF-NATO Postdoctoral Fellowship for research at the University of Cambridge	$23,600

GRANTS IN SUPPORT OF CONFERENCES

M. D. Hollingsworth and J. R. Scheffer April, 1991	Petroleum Research Fund (Type SE), "Tenth International Conference on the Chemistry of the Organic Solid State"	$2,000
M. D. Hollingsworth and J. R. Scheffer April, 1991	NSERC, Canada (Conference Grant) "Tenth International Conference on the Chemistry of the Organic Solid State"	CAN$2,000

In 1991, I raised money from the following companies for the Tenth International Conference on the Chemistry of the Organic Solid State: Bruker Spectrospin Canada: Can$500, Chemagnetics, Inc.: $250, Doty Scientific, Inc.: $500, GTE Laboratories: $1000. In 1995, I raised $500 from Eli Lilly, Inc. for the Seventh Midwest Organic Solid State Chemistry Symposium. In 2008, I raised $500 from TransForm Pharmaceuticals and $500 from Ipharma for the 19th Midwest Organic Solid State Chemistry Symposium. In 2017, I raised $250 from Crystal Growth and

Design and $250 from Bruker Spectrospin, Inc, for the 27th Midwest Organic Solid State Chemistry Symposium.

OTHER EXPERIENCE:

GOLDWATER SCHOLARSHIP NOMINATING COMMITTEE: At Kansas State University, I joined the Goldwater Scholarship nominating and advisory committee in 2017.

CO-CHAIR: Twenty-seventh Midwest Organic Solid State Chemistry Symposium, Manhattan, KS, June 9-10, 2017.

CO-CHAIR (with Bart Kahr): Symposium on "Fundamentals of Crystal Growth," at the 18th American Conference on Crystal Growth and Epitaxy, July 31 – Aug. 5, 2011, Monterey, CA.

GROW WORKSHOPS FOR MIDDLE SCHOOL GIRLS: In the summers of 2005, 2007, 2008, 2010, and 2011, I have conducted GROW (Girls Researching Our World) workshops ("This View of Crystals") on growth of ferroelastic crystals, ferroelastic domain switching of urea inclusion compounds and crystal optics. This very successful workshop was part of a larger effort of the Women in Engineering and Science Program (WESP) at KSU to attract 11-13 year old girls to careers in science.

CO-CHAIR: Nineteenth Midwest Organic Solid State Chemistry Symposium, Manhattan, KS, June 13-14, 2008.

GUEST PROFESSOR: In the summer of 2005, I was a guest lecturer at a "Summer School" on "Stereochemical Aspects of Novel Materials," for U.S. and Latin American scholars at the Santa Barbara International Center for Materials Research. The course was held on the UCSB campus and was attended by students from UCSB, UCLA and Latin America.

GUEST EDITOR (with Profs. Bart Kahr and Jennifer Swift) of a special issue of *Crystal Growth and Design* in honor of the 65th year of Prof. J. Michael McBride. This issue appeared in Nov. 2005.

VISITING PROFESSORSHIPS: During the summers of 2000 and 2001, 2006, 2007, 2009, 2010, 2012, 2014, the fall of 2008, and in the spring and summer of 2005, I have been a Visiting Professor in the Condensed Matter and Materials Group in the Department of Physics at the University of Rennes in Rennes, France. I have also been a Visiting Professor in the Department of Chemistry at the University of Bordeaux in July of 2006. In 2018, I have been a CNRS Visiting Professor at the University of Rennes.

MEMBER – PARTICIPATING RESEARCH TEAM: I have been a member of the Participating Research Team (PRT) at the Stony Brook Topography Facility, Beamline X-19C at the National Synchrotron Light Source at Brookhaven National Laboratory since 2000.

CHAIR – COLLEGE ENVIRONMENTAL HEALTH AND SAFETY COMMITTEE: In 2004, I chaired a committee of six faculty members that initiated and oversaw a waste audit that was mandated by the EPA as part of a consent agreement to reduce a substantial fine from KSU for violations of the Resource Conservation and Recovery Act. This very successful audit covered almost every room in the College of Arts and Sciences.

RHODES/MARSHALL SELECTION COMMITTEE: At Kansas State University, I have been serving on the Rhodes/Marshall Scholar selection and advisory committee (2002-present).

INFORMATION RESOURCE MANAGEMENT COUNCIL, KANSAS STATE UNIVERSITY: This committee advises the Provost on policies regarding all aspects of technology in dissemination, storage and retrieval of information at the university (2005 to 2008).

WOMEN IN ENGINEERING AND SCIENCE ADVISORY COMMITTEE: I served on the search committee for the Director of the Women in Engineering and Science Program at Kansas State University and subsequently on the advisory committee for that office (1999-2003).

GEOLOGY AND PHYSICS FACULTY SEARCH COMMITTEE: In addition to serving on numerous search committees in chemistry, I have been the outside member of a search committee for a mineralogist/petrologist in the Geology Dept. and a nanobiophysicist in the Physics Dept.

EXPERT CONSULTANT: Since 1997, I have been retained as an expert consultant and expert witness for pharmaceutical companies on several patent cases involving polymorphism and pseudomorphism of crystalline pharmaceuticals and the reactions used to make them. During this time, I have appeared at trial three times. I have also served as an expert consultant for TransForm Pharmaceuticals.

CO-CHAIR: Seventh Midwest Organic Solid State Chemistry Symposium, Bloomington, IN, June 9-10, 1995.

SPECIAL ISSUE OF CHEMISTRY OF MATERIALS: With Michael D. Ward (University of Minnesota), I instigated and coordinated a special issue of *Chemistry of Materials* in honor of the late Margaret C. (Peggy) Etter. Prof. Ward and I selected authors for review articles, refereed a large number of the papers, wrote the introductory material for the issue and sequenced the articles, reviews and communications. This was an extremely successful issue that received wide attention.

GUEST EDITOR (with John Scheffer) of a two-volume (~650 page) issue of *Molecular Crystals and Liquid Crystals:* The Proceedings of the Tenth International Conference on the Chemistry of the Organic Solid State

FACULTY SALARIES AND PROMOTIONS COMMITTEE (FACULTY OF SCIENCE, UNIVERSITY OF ALBERTA) 1989-91. Evaluated approximately 300 faculty dossiers each year for salaries and promotion.

CO-CHAIR: Tenth International Conference on the Chemistry of the Organic Solid State (ICCOSS X), Vancouver, B. C., July 1991.

INTERNATIONAL SCIENTIFIC COMMITTEE: International Conference on the Chemistry of the Organic Solid State: (ICCOSS IX), Como, Italy, July, 1989; (ICCOSS X), Vancouver, British Columbia, July, 1991; (ICCOSS XI), Jerusalem, Israel, July, 1993; (ICCOSS XII) Matsuyama, Japan, July 1995; (ICCOSS XIII) Stony Brook, New York, July, 1997; (ICCOSS XIV) Cambridge, U.K, July 1999; (ICCOSS XV) Mainz, Germany, July, 2001; (ICCOSS XVI) Sydney, Australia, July, 2003; (ICCOSS XVII) Los Angeles, CA, July 2005; (ICCOSS XVIII) Merida, Venezuela, July 2007; (ICCISS XIX) Sestri Levante, Italy, June 2009; (ICCOSS XX), Bangalore, India, June, 2011, (ICCOSS XXI), Oxford, August, 2013, (ICCOSS XXII), Niigata, Japan, July, 2015, (ICCOSS XXIII), Stellenboch, South Africa, 2017, New York, NY, 2019 ((ICCOSS XXIII).

REFEREEING - I have done extensive refereeing for scientific journals (including Science, Nature, Nature Chemistry, Journal of the American Chemical Society, Angewandte Chemie, Chemical Communications, Chemistry of Materials, Journal of Pharmaceutical Sciences, Journal of Inclusion Phenomena and Macrocyclic Chemistry, Organometallics, Tetrahedron Letters, Inorganic Chemistry, Journal of Materials Chemistry, Chemical Reviews, Proceedings of the Royal Society, Section A (Math and Phys. Sci.), Journal of Physical Chemistry, Journal of Organic Chemistry, Acta Crystallographica, Tetrahedron, Journal of Physical Organic Chemistry, Macromolecules, Molecular Crystals and Liquid Crystals, Crystal Growth and Design, CrystEngComm, Organic Letters, Advanced Functional

Materials, Journal of Computational Chemistry, Journal of Solid State Chemistry, Molecular Pharmaceutics, Journal of Molecular Structure, Journal of Chemical Theory and Computation, New Journal of Chemistry, Langmuir) and for granting agencies (including the National Science Foundation, Petroleum Research Fund, United States-Israel Binational Science Foundation, Department of Energy, Alzheimer's Association, the Research Corporation, Unversity of Padova Young Scholars Program, Swiss National Science Foundation, Intense Pulsed Neutron Source (Argonne National Laboratory),). As part of the latter, I have been a panelist for the Major Research Instrumentation program at the National Science Foundation. (This was under the Chemistry Research Instrumentation and Facilities program and focused on high field NMR proposals.)

TEACHING: At the University of Alberta, I taught introductory organic chemistry to freshmen and a graduate course in organic structural analysis. At Indiana University, I taught a graduate/undergraduate course in spectroscopic methods of structure determination, a graduate course in physical organic chemistry, introductory organic chemistry, first semester organic laboratory, second semester organic laboratory, and honors organic chemistry I and II. At Kansas State University, I have taught a freshman tutorial program, Physical Methods in Inorganic Chemistry, Organic Chemistry I and II, Organic Chemistry Laboratory, General Organic Chemistry, Advanced Organic Chemistry Laboratory, Advanced Organic Chemistry, Physical Organic Chemistry, Organic Spectroscopy, six lectures on tensorial properties of crystals for a team-taught course on Materials Chemistry and the departmental Ethics Seminar for incoming graduate students.

CHEMICAL WEAPONS RISK ASSESSMENT: Because of my calculations and assessment of the public health risk of open-air testing of nerve gases at Defence Research Establishment Suffield in 1988, the Canadian government suspended further outdoor testing of these agents.

THE NERVE CENTER: As a graduate student, I helped set up a disarmament organization that focused on disseminating information about chemical and biological weapons. When I moved to England as a postdoctoral student, I continued this work with The Working Party on Chemical and Biological Weapons, a London based defense information/disarmament group.

YALE RECYCLING: Played a major role in the initiation and development of a very successful university-wide recycling program, with over 100 sites in 60 buildings. Negotiated with administration officials at most levels for university support of this recycling program.

CARLETON RECYCLING: Initiated and coordinated a campus-wide recycling program.

NATURALIST: Served as a naturalist for the Carleton Arboretum and for the Branford Land Trust. On three occasions, I helped my ex-wife lead tourist groups on one-week trips to the Peruvian Amazon. Between 1998 and 2003, I also served as a staff member on three of her Earthwatch Expeditions, which focused on bird conservation in a remote cloud forest in Southwestern Ecuador.

HOBBIES AND INTERESTS: bird watching and vocalizations, photography, bicycling, natural history, and conservation.

EXHIBIT C

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High-performance liquid chromatographic analysis of chlorhexidine phosphanilate, a new antimicrobial agent*

R. RAO GADDE,† EDWARD F. McNIFF and MARYANN M. PEER

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Abstract: Chlorhexidine phosphanilate (CHP) is analysed by two separate reversed-phase HPLC methods. CHP was found to be a non-stoichiometric compound with a phosphanilic acid to chlorhexidine ratio of 1.83. By careful choice of solvents, solution pH and HPLC columns, loss of sample due to incomplete dissolution and adsorption to surfaces is avoided. Both methods are shown to be stability-indicating and accurate.

Keywords: *Chlorhexidine phosphanilate; chlorhexidine; phosphanilic acid; HPLC; stoichiometry; stability; chlorhexidine acetate.*

Introduction

Chlorhexidine phosphanilate (CHP) is a salt of chlorhexidine and phosphanilic acid (Fig. 1). It is a non-stoichiometric compound with a chlorhexidine to phosphanilic acid ratio of 1.83 and is amorphous in nature. In solution it dissociates into chlorhexidine and phosphanilate ions. It has a remarkably broad spectrum of antibacterial activity encompassing the predominantly gram-positive spectrum of chlorhexidine and the broad gram-negative activity of phosphanilic acid [1]. Its lack of cross resistance with sulfonamide resistant strains [2] makes it especially attractive for burn wound treatment. The broad spectrum activity of this compound suggests potential applications for prevention and treatment of skin infections. The release of CHP from a cream vehicle and its skin permeation characteristics has been reported [3].

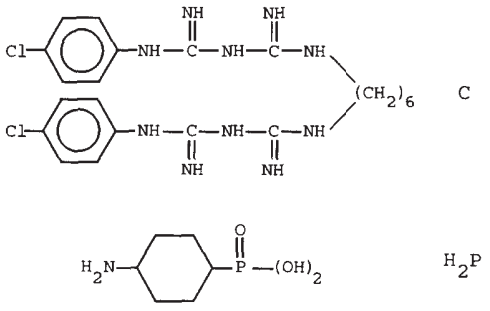

Figure 1
Chemical structures of chlorhexidine (C) and phosphanilic acid (H_2P).

CHP is practically insoluble in most common solvents with the exception of dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF). Its solubility in water is about 0.4 mg ml^{-1}. Chromatographically, it is observed as its two component ion peaks, chlorhexidine and phosphanilate, and not as a single CHP peak. Since both chlorhexidine and phosphanilate are therapeutically active and because CHP is a non-stoichiometric compound, it is important to monitor both chlorhexidine and phosphanilate during the development of the drug. Because of their difference in polarity and the ionic interactions of the chlorhexidine and phosphanilate ions, it is difficult to develop a single HPLC method for both ions.

A number of HPLC methods for chlorhexidine have been reported and a good bibliography of these and other analytical methods may be found in refs 4–6. Most of these HPLC methods use an octadecylsilane (C_{18}) column and a mobile phase of aqueous methanol or acetonitrile containing an ion-pairing agent. Only one HPLC method was reported in the literature for phosphanilic acid [7]. Direct application of any of these methods

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†Author to whom correspondence should be addressed.

to CHP analysis is not possible because of the poor solubility of CHP in water and in most other solvents used in HPLC mobile phases. In addition, phosphanilic acid is also very sparingly soluble in water and it is insoluble or sparingly soluble in the usual organic solvents [8]. The problems associated with low solubilities are losses due to incomplete dissolution, precipitation and surface adsorption. These problems have been circumvented in the two individual HPLC assay methods reported in this paper. Practical details for easy application of these methods, along with method development and validation information, are presented.

Experimental

Instrumentation

A Waters Associate (Milford, MA, USA) chromatograph equipped with Model 6000A pump, WISP Model 710 injector, Model 440 absorbance detector (254 nm) was used. A Hewlett–Packard (Palo Alto, CA, USA) Model 1040A diode array spectrophotometric detector was used as needed to simultaneously monitor the chromatogram and absorption spectra of the component peaks. Data acquisition and integration of peaks were performed with a Hewlett–Packard Model 3352D Laboratory Automation System.

For chlorhexidine assay, a Waters μBondapak C_{18} column (30 cm × 3.9 mm i.d.) was used. A Waters μBondapak-NH_2 column was used in phosphanilic acid analysis. The eluent flow rate was 1.0 ml min^{-1} for the former analysis and 2.0 ml min^{-1} for the latter. In both cases, an injection volume of 10 μl was used.

Reagents and solvents

Chlorhexidine acetate (Sigma, St Louis, MO, USA) was used as the chlorhexidine standard. Its purity was assessed by non-aqueous titration with 0.1 M perchloric acid in glacial acetic acid and its water content by Karl Fischer titration. The purity of phosphanilic acid (Bristol–Myers Squibb Co., Syracuse, NY, USA) was determined by potentiometric titration with nitrite. CHP was manufactured under Bristol–Myers Squibb control. Ethyl benzoate was purchased from Aldrich (Milwaukee, WI, USA).

Tetrahydrofuran (THF), acetonitrile, 85% m/m phosphoric acid and ammonium dihydrogen phosphate were all of HPLC grade (Fisher Scientific, Pittsburgh, PA, USA). All other solvents and reagents were of ACS grade from Fisher Scientific. The water used was deionized and organic free quality from Milli Q System (Millipore, Bedford, MA, USA).

Mobile phase

For the chlorhexidine analysis, the mobile phase was 40:10:50 (v/v/v) mixture of methanol, THF and 0.1 M aqueous sodium sulphate, the pH of which was adjusted to 2.2 with sulphuric acid. The mobile phase for the phosphanilic acid analysis was a 10:90 mixture of acetonitrile and 0.012 M aqueous ammonium dihydrogen phosphate.

Standards preparation

The chlorhexidine standard for HPLC analysis was prepared to contain 0.04 mg ml^{-1} chlorhexidine acetate and 0.33 mg ml^{-1} ethyl benzoate (internal standard) using methanol as the solvent.

Separate stock solutions of phosphanilic acid (1 mg ml^{-1}) and salicylic acid (14 mg ml^{-1}) were prepared in 0.5 N sodium hydroxide solution to facilitate easy dissolution. To prepare the standard, phosphanilic and salicylic acid stock solutions, 4 and 5 ml respectively, were mixed with 9 ml of dilute phosphoric acid (4.34% w/v) and the mixture diluted to 100 ml with 45:55 (v/v) acetonitrile–water mixture. This sample preparation resulted in the diluent being approximately equivalent to the mobile phase so as to minimize baseline disturbances.

Sample preparation

The solution of CHP sample (2.5 mg ml^{-1}) was prepared in DMSO. For the phosphanilic acid assay, 4 ml of this sample solution was mixed with 5 ml of the salicylic acid stock solution (see standard preparation above) and 5 ml of dilute phosphoric acid (4.34% w/v) solution. The resultant solution was diluted to 100 ml with 45:55 (v/v) acetonitrile–water mixture.

For the chlorhexidine assay, the CHP sample solution (2.5 mg ml^{-1}) in DMSO was diluted five-fold with DMSO. The sample for analysis was then prepared from this dilute solution to contain 0.05 mg ml^{-1} CHP and 0.33 mg ml^{-1} ethyl benzoate using methanol as the diluent.

Results and Discussion

Solubility and solution equilibria

The solubility of CHP depends on the solvent, pH, temperature and the counter ions in solution. It is more soluble in DMSO (over 100 mg ml^{-1}) and DMF than in water, methanol, 1-propanol, 2-propanol and acetonitrile. In solution, CHP dissociates into chlorhexidine and phosphanilate ions which in turn exist in equilibrium with their multiple acid and base forms depending on the pH of the solution.

Chlorhexidine can exist as free base (C) or its protonated forms CH_2^{2+} and CH_4^{4+}. pK_a values are reported to be 2.2 and 10.3 [9, 10]. Although only one pK_a value of 7.5 is reported for phosphanilic acid [11] it may exist as its protonated form, free acid and mono- and dianionic forms. The approximate pK_a values are estimated from pH titration curves and solubility in strongly acidic solution to be <0.5, 3.5 and 7.1 (C. Zusi, personal communication). Depending on the pH of the medium, different ionic species form in CHP solutions. A schematic view of the equilibria in aqueous solution is shown in Fig. 2. The vertical lines represent points where pH = pK_a. pH regions where the individual species are predominant are shown by horizontal solid lines. The solubility and dissolution rate of CHP are pH-dependent and they become more complex if the pH is favourable to the formation of other sparingly soluble salts (e.g. chlorhexidine monophosphanilate), phosphanilic acid (free acid) and/or chlorhexidine (free base). In water, the solubility of chlorhexidine is only 0.08 mg ml^{-1} [12] and phosphanilic acid is known to be very sparingly soluble [8]. The common salts of chlorhexidine (e.g. chloride, nitrate, sulphate) have solubilities in the range 0.1–1 mg ml^{-1} [12], hence the presence of simple anions could play a significant role in the dissolution kinetics of CHP. Furthermore, the poor solubility of chlorhexidine, phosphanilic acid and related ionic species and salts may be contributory factors to adsorptive losses during sample preparation and analysis.

Analytical standards

Early in the method development it was considered using well characterized lots of CHP as the reference standard for both chlorhexidine and phosphanilic acid assays by HPLC. However, it was realized later that CHP is not a stoichiometric compound and that more reliable standards of chlorhexidine and phosphanilic acid were needed.

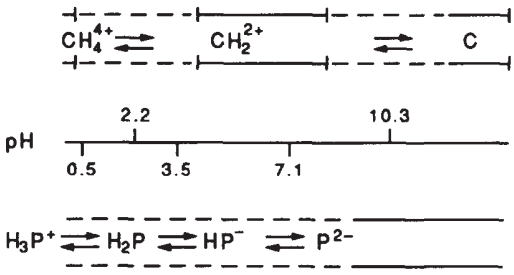

Figure 2
pH-dependent equilibria of chlorhexidine and phosphanilic acid in aqueous solutions.

Among the chlorhexidine salts, chlorhexidine acetate was selected as the analytical standard. It is commercially available in pure form and can be easily purified further by recrystallization from water. Typically, it assayed better than 99% (after correction for water) by non-aqueous titration with 0.1 N perchloric acid in glacial acetic acid [13]. Our data show that it contained about 3.2% m/m moisture (Karl Fischer titration) but it is not hygroscopic at 80% relative humidity. Phosphanilic acid was available at 98.5% m/m purity as shown by titration with nitrate [14], and by HPLC (peak area normalization). Thus a suitable correction was applied in calculations.

Chromatography development

In reversed-phase chromatography with μBondapak C_{18} column, CHP behaved as a mixture of two components (chlorhexidine and phosphanilic acid). With weak mobile phases, only one peak due to phosphanilic acid was observed at or near the solvent front. With stronger mobile phases, chlorhexidine peak also showed up generally as a broad peak. It was impossible to retain phosphanilic acid while eluting chlorhexidine in a reasonable time. Similar behaviour of CHP was also observed using μBondapak phenyl column. Only with mobile phases of low pH (about 2.5) did chlorhexidine show a sharp peak. At pH 2.5, which was needed for the optimal peak shape of chlorhexidine, it was not possible to move the phosphanilic acid peak away from the solvent front by ion-pairing. Hence, it became necessary to develop separate assay methods for chlorhexidine and phosphanilic acid.

The best mobile phase for chlorhexidine using μBondapak C_{18} column, was methanol–

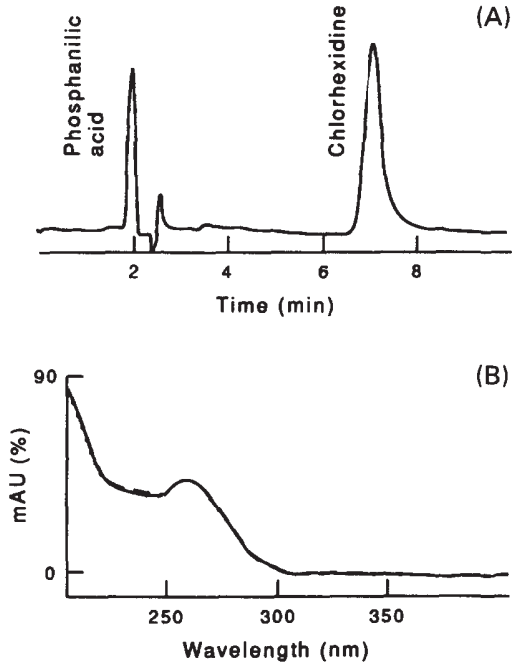

Figure 3
HPLC chromatogram (A) of chlorhexidine phosphanilate and UV spectra (B) at the upslope, apex and downslope of chlorhexidine peak.

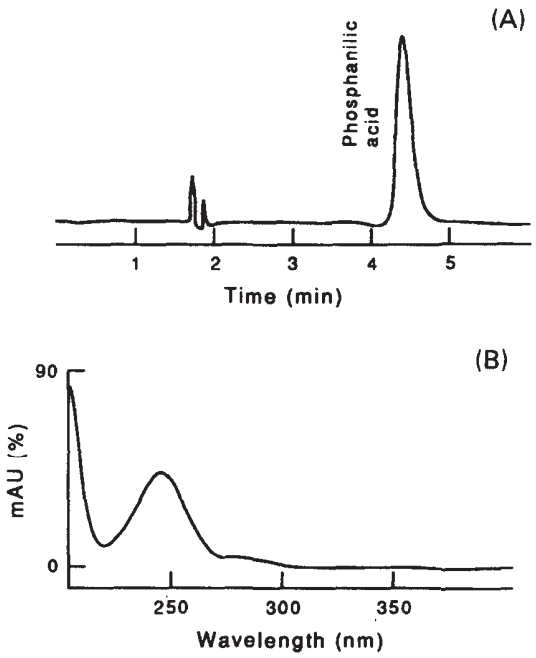

Figure 4
HPLC chromatogram (A) of chlorhexidine phosphanilate and UV spectra (B) at the upslope, apex and downslope of phosphanilic acid peak.

THF–0.1 M sodium sulphate (4:1:5, v/v/v), with the pH adjusted to 2.5 with sulphuric acid (Fig. 3). The use of methanol, instead of acetonitrile, is advantageous since CHP is more soluble in methanol (>0.14 mg ml^{-1}) than in acetonitrile. The addition of THF and sodium sulphate resulted in better separation of chlorhexidine from its impurities. The inclusion of THF as a mixed organic solvent system improved the peak shape. Sodium sulphate acts as an ionic suppressor.

Phosphanilic acid gave a peak with minimal tailing and a reasonable retention time using μBondapak amine column (Fig. 4). A simple mobile phase of acetonitrile–0.012 M ammonium dihydrogen phosphate buffer (1:9, v/v) and the absence of ion-pairing agents gave good separation of phosphanilic acid and the internal standard, salicylic acid. An increase in resolution was observed with lower phosphate concentration but with a corresponding increase in retention times and analysis time. However, by lowering the phosphate concentration and increasing acetonitrile concentration, the useful life of the amine column can be extended especially for CHP product analysis where potential deactivation of the column can be caused by excipients present in the formulation.

The standards and samples for injection for both chlorhexidine and phosphanilic acid methods were matched approximately to have a similar medium (solvents, pH) which is compatible with the mobile phase. Because of the complex equilibria which exist in CHP solutions and the poor solubilities of several components in equilibria, this matching is considered important. A similar observation was made by others [5, 6] in the assay of chlorhexidine gluconate in ophthalmic solutions. The solvents, pH, concentrations (chlorhexidine, phosphanilic acid, CHP) and the sequence of dilutions were selected in a manner to minimize any losses due to precipitation or adsorption on surfaces. Sudden perturbations in equilibria due to sudden changes in pH and concentrations of counter ions could lead to loss of analyte or distortion of the peaks in chromatographic separations.

Chlorhexidine method validation

Using solutions of chlorhexidine acetate standard with the internal standard (ethyl benzoate), the linearity of the chlorhexidine

response ratio was studied. Both peak height and peak area ratios showed good linear response in the chlorhexidine concentration range 0.013–0.053 mg ml^{-1} (correlation coefficient >0.999). A low negative *y*-intercept by peak height ratio, 2.3% compared to response at 0.033 mg ml^{-1} chlorhexidine, suggests that a single point standard may be used in the assay instead of a calibration curve with multiple standards. The *y*-intercept by peak area ratio was somewhat higher (5.1%).

Specificity of the method was demonstrated by analysing methanolic solutions of CHP (0.27 mg ml^{-1}) which were force-degraded by heat (60°C) and light (≈1000 foot candles). After 8 days, the chlorhexidine content was found to be 13.0 and 94.9% m/m of the initial in the heat and light degraded samples respectively. No peaks interfering with either the chlorhexidine or the internal standard peak were observed (Fig. 5). *p*-Chloroaniline, a known hydrolytic degradation product of chlorhexidine [15, 16] did not interfere. It elutes on the tail end of the phosphanilic acid peak near the solvent front.

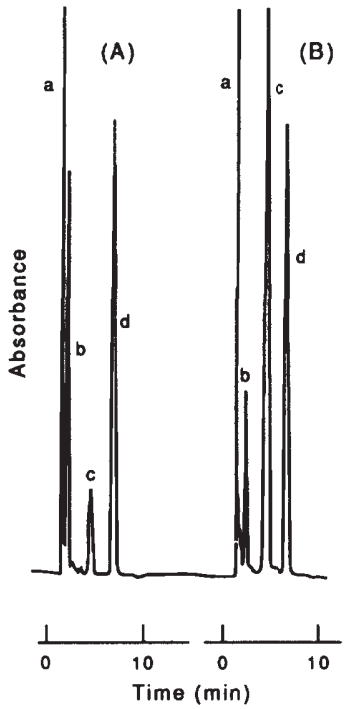

Figure 5
Chromatograms of degraded chlorhexidine phosphanilate solutions by the chlorhexidine assay method. (A) Heat degraded solution (8 days, 60°C). (B) Light degraded solution (8 days, 1000 foot candles). Peak identification: a, phosphanilic acid; b, degradation product; c, chlorhexidine and d, ethyl benzoate.

Purity of the chlorhexidine peak was studied by recording the spectral–chromatographic data using the photodiode array detector. A typical superposition of upslope, apex and downslope spectra is shown in Fig. 3. Good superimposability of spectra from three regions of the peak and also with that of chlorhexidine (acetate) standard provided evidence for the absence of coeluting peak(s) under the chlorhexidine peak.

Four solutions of chlorhexidine acetate spiked with different amounts of sodium phosphanilate were analysed by the chlorhexidine method. Chromatographic responses (chlorhexidine to internal standard peak area ratio) of 1.134, 1.140, 1.120 and 1.120 were observed when the phosphanilic acid to chlorhexidine molar ratio in solutions were 0.88, 1.40, 1.75 and 2.10, respectively. These data demonstrate that the chlorhexidine response is not sensitive to the phosphanilic acid to chlorhexidine ratio.

Phosphanilic acid method validation

The response ratio (phosphanilic acid to salicylic acid peak area ratio) was observed to be linear in the concentration range 0.02–0.06 mg ml^{-1} of phosphanilic acid. A correlation coefficient of 0.9999 and a *y*-intercept of 0.006% (compared to response ratio of 0.04 mg ml^{-1} phosphanilic acid standard) were calculated.

CHP solutions degraded by light and heat, using conditions described under chlorhexidine method validation, were analysed by the phosphanilic acid assay method. Chromatograms in Fig. 6 show the separation of both phosphanilic acid and salicylic acid peaks from the degradation products of CHP. The purity of the phosphanilic acid is demonstrated by the essentially identical spectra recorded on the upslope, apex and downslope portion of the peak (Fig. 4).

The effect of chlorhexidine on the phosphanilic acid response was also studied by analysing sodium phosphanilate solutions spiked with chlorhexidine acetate. Analysis of four solutions with molar ratios of phosphanilic acid to chlorhexidine that varied from 2.06 to 4.96 showed no effect on the phosphanilic response (0.13% RSD).

Composition of chlorhexidine phosphanilate

Sixteen lots of CHP drug substance were analysed separately for their chlorhexidine and phosphanilic acid content. The molar ratio of

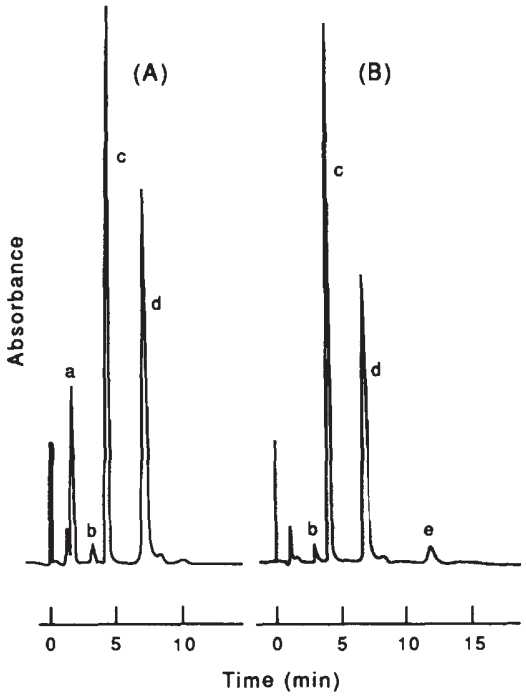

Figure 6
Chromatograms of degraded chlorhexidine phosphanilate solutions by the phosphanilic acid assay method. (A) Heat degraded solution (18 weeks, 60°C). (B) Light degraded solution (18 weeks, 1000 foot candles). Peak identification: a, degradation product; b, system peak; c, salicylic acid; d, phosphanilic acid and e, degradation product.

phosphanilic acid to chlorhexidine was found to be in the range of 1.76–1.88 with a mean of 1.83 (n = 16, RSD = 2.2%). The corresponding molar ratios obtained from elemental analysis data (chlorine and phosphorus assays) are in the range 1.77–2.01 with a mean of 1.92 (RSD = 3.9%). Considering the normal uncertainty in the elemental analysis data, the ratios observed by the two techniques are considered to be in fair agreement. Hence the CHP drug substance is a non-stoichiometric compound with a mean phosphanilic acid to chlorhexidine molar ratio of 1.83.

Conclusions

The complex equilibria of CHP in solution and the poor solubility of CHP and its equilibration products required special care in preparing and handling of samples and standards. Analysis of CHP by two separate stability-indicating HPLC methods for chlorhexidine and phosphanilic acid demonstrated that CHP is a non-stoichiometric compound with phosphanilic acid to chlorhexidine ratio of 1.83.

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A HILIC method for the analysis of tromethamine as the counter ion in an investigational pharmaceutical salt

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Abstract

A hydrophilic interaction chromatography (HILIC) method using an aminopropyl stationary phase was developed and validated to determine the content of tromethamine as counter ion in an investigational drug substance. Tromethamine was a very polar compound without any chromophores, and could not be readily retained and detected by conventional reserved-phase HPLC-UV methods. Furthermore, the tromethamine salt of the drug compound also had limited solubility in aqueous solution. The method employed simple acetonitrile/water mobile phase (80/20, v/v) to provide sufficient retention for tromethamine. Meanwhile, the high acetonitrile content also helped to dissolve the drug substance sample. Refractive index (RI) was used for the detection of tromethamine. The method was found to be specific without interference from the drug compound and related impurities. The method was also validated for suitable precision, linearity and accuracy for the analysis of drug substance samples. The effects of various parameters, such as acetonitrile content, mobile phase salt concentration, and column temperature on HILIC separation were investigated.

Keywords: Tromethamine; HILIC; Pharmaceutical salt

1. Introduction

2-Amino-2-hydroxymethyl-propane-1,3-diol (tromethamine) is a weak base with pK_a ~8.1 (25 °C) and is readily soluble in water [1]. It is commonly used as a buffering or emulsifying agent in pharmaceutical and cosmetic products, or as a counter ion for acidic pharmaceutical compounds to form desired salt forms. When used as an ingredient in pharmaceutical preparations, suitable analytical methods are required to establish its identity and quantity for quality control and regulatory purposes. Chromatographic methods have been developed for the determination of tromethamine in biological fluids and pharmaceutical products [2–4]. Two reverse-phase HPLC methods involved chemical derivatization to provide sufficient retention on HPLC columns as well as chromophores or fluorophores for UV or fluorescence detection [2,3]. An ion chromato-

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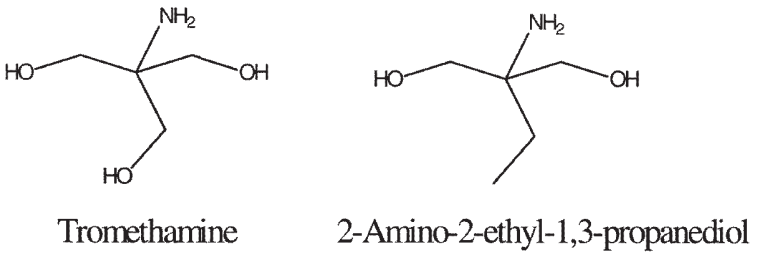

Tromethamine 2-Amino-2-ethyl-1,3-propanediol

Fig. 1. Structures for tromethamine and 2-amino-2-ethyl-1,3-propanediol (AEPD).

graphic (IC) method developed by Hall et al. avoided chemical derivatization and was used to analyze tromethamine in Alomide® ophthalmic solution (as counter ion to lodoxamide) [4]. More recently, a CE method was also developed to analyze tromethamine in a contact-lens cleaning solution (as buffering agent) [5]. The IC and CE procedures were very straightforward. These methods, however, required that pharmaceutical dosage forms or drug substance be readily soluble in aqueous solution.

An investigational agent for treating asthma is currently under development in our company, and the active pharmaceutical ingredient (API) is prepared as a tromethamine salt. An analytical method is needed to quantitate the amount of tromethamine as the counter ion in the API and also to determine the stoichiometry of the salt form. A simple and accurate analytical method is preferred for drug substance release testing. However, the IC or CE method is not readily applicable in our case due to the fact that the non-salt form (free acid) of the API under investigation is not water soluble, and even its tromethamine salt has only limited solubility in water. Therefore, an alternative chromatographic method for the analysis of tromethamine in the API is needed.

Tromethamine is a very polar compound with three hydroxyl groups as shown in Fig. 1. No retention can be attained for tromethamine on reverse-phase columns even using pure aqueous mobile phases (data not shown). An alternative approach to obtain sufficient retention in chromatographic separations for very polar compounds is hydrophilic interaction chromatography (HILIC). HILIC is often considered as a 'normal phase separation' in a reversed-phase fashion wherein separations occur on polar stationary phases (e.g. silica or aminopropyl phase) with aqueous–organic mobile phases. In contrast to reversed-phase chromatography, polar compounds have stronger retention than non-polar compounds [6]. This mode of chromatography has been used mostly in the area of sugar, nucleic acids and peptide analysis [6–8]. More recently, several literature reports demonstrate the application of HILIC for the determination of polar pharmaceutical compounds [9–11].

In this study, a HILIC method using an aminopropyl column, acetonitrile/water mobile phase, and RI detection is described. Detailed consideration for method development is discussed as well as various parameters affecting the HILIC separation. The applicability of the HILIC method is demonstrated by analyzing tromethamine salt samples of the investigational drug and determining the stoichiometry of the salt form.

2. Experimental

2.1. Apparatus

All the experiments were performed on an Agilent HP1100 HPLC system equipped with both diode array (DAD, Model G1315A) and refractive index (RI, Model G1362A) detector (Agilent Technologies, Polo Alto, CA). The HPLC system also included a quaternary pump (Model G1311A), a degasser (Model G1322A), a column heater (Model G1316A), and an auto-injector (Model G1313A). The DAD and RI detectors were connected in series. The chromatograms were recorded using Agilent Chemstation

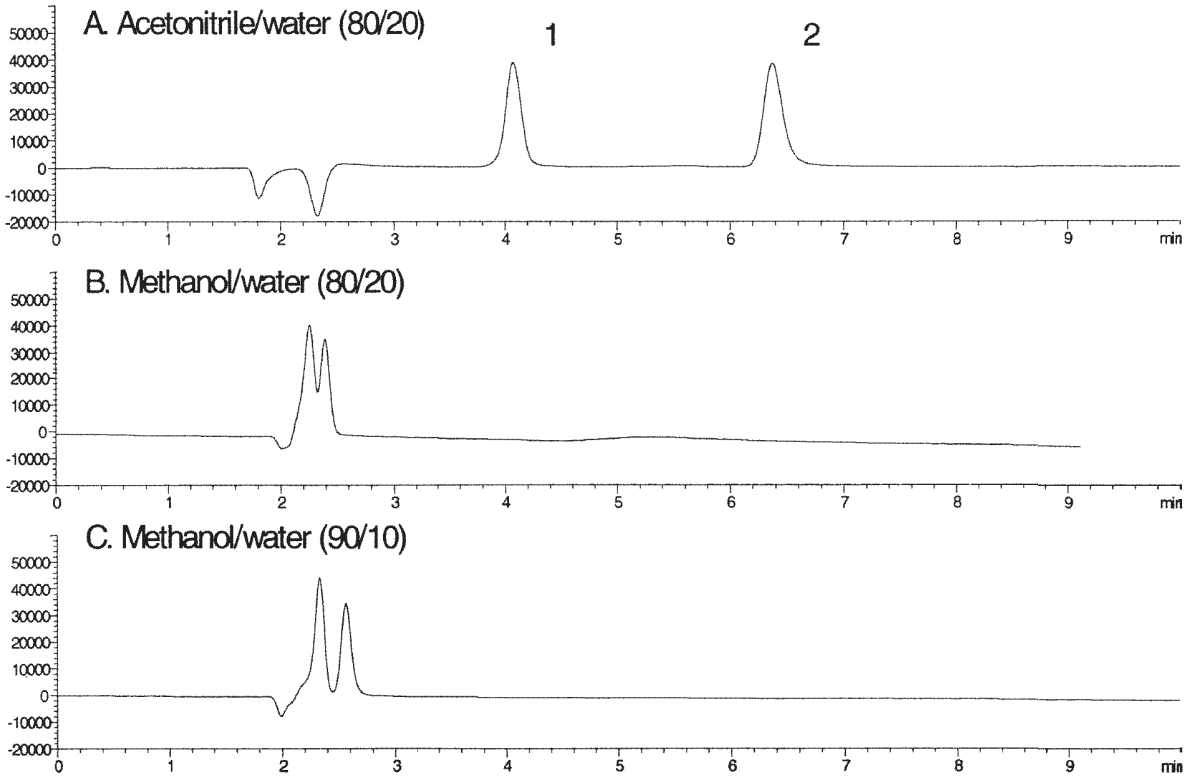

Fig. 2. Chromatograms for the separation of AEPD (Peak 1) and tromethamine (Peak 2) using the mobile phase (A) acetonitrile/water (80/20), (B) methanol/water (80/20) and (C) methanol/water (90/10). Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size. Column temperature: 25 °C. Flow rate: 1 ml/min. Samples: AEPD and tromethamine standards (~1 mg/ml) each in the mobile phase. Injection volume: 50 μl.

software (Rev. A. 09. 01). The following columns were used in the experiments: Zorbax NH_2, 70 Å pore diameter from Agilent Technologies (Wilmington DE), Nucleosil NH_2, 100 Å pore diameter from Phenomenex (Torrance, CA), YMC-Pack NH_2, 120 Å pore diameter from Waters (Milford, MA). All the columns were 150 × 4.6 mm I.D. with 5 μm particles.

2.2. Reagents

HPLC grade acetonitrile, methanol and isopropanol (IPA) were purchased from Burdick & Jackson (Muskegon, MI). Ammonium acetate (HPLC grade) was obtained from J. T. Baker (Phillipsburg, NJ). Tromethamine standard (+ 99.9%) was purchased from Aldrich (Milwaukee, WI), and 2-amino-2-ethyl-1,3-propanediol (AEPD) was from Acros (New Jersey, USA).

2.3. Chromatographic conditions

All the columns were washed with IPA for at least 30 min at 1 ml/min to remove the storage solvent (hexane), then with acetonitrile/water mixture (50/50, v/v) for about 1 h, and finally equilibrated with the mobile phase. The mobile phase was composed of only acetonitrile and water (80/20, v/v) for the final method. All reference standards and samples were dissolved in the mobile phase. The optical unit temperature of the RI detector was set at 35 °C to minimize baseline noise. The flow rate was 1.0 ml/min and injection volume was 50 μl for the method.

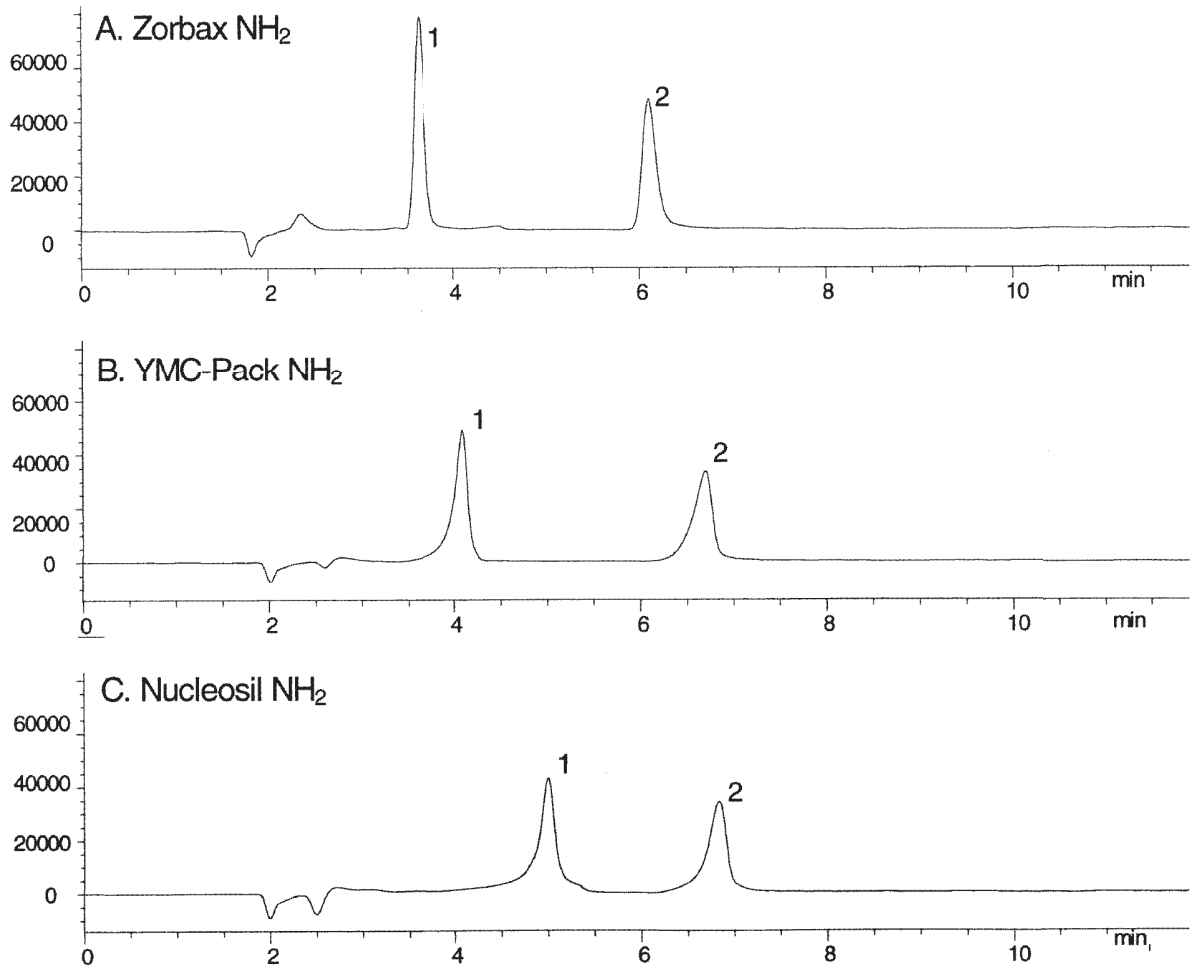

Fig. 3. Chromatograms for the separation of AEPD (Peak 1) and tromethamine (Peak 2) on three amino columns, (A) Zorbax NH_2, (B) YMC-Pack NH_2, and (C) Nucleosil NH_2 column. Column temperature: 25 °C. Mobile phase: acetonitrile/water (80/20, v/v). Flow rate: 1 ml/min. Samples: AEPD and tromethamine standards (~ 1 mg/ml) each in the mobile phase. Injection volume: 50 μl.

3. Results and discussion

3.1. Method development

The HILIC approach was employed for tromethamine separation based on the fact that it had been shown to provide sufficient retention for very polar compounds such as sugars, uracil, acetamide, etc. [6,7,9]. In addition to tromethamine, another structurally related compound, AEPD was also selected to aid method development. In this study, only amino stationary phase was used and the mobile phase was a simple mixture of water and organic solvent (i.e. acetonitrile and methanol). Fig. 2 shows the separation of tromethamine and AEPD on a Zorbax NH_2 column using acetonitrile/water or methanol/water mobile phase. Under the experimental conditions, the more hydrophilic tromethamine eluted after AEPD, which was expected for the HILIC separation. Sufficient retention was obtained using the acetonitrile/water (80/20, v/v) mobile phase ($k' \sim 2.3$ for tromethamine); however, the retention was greatly reduced in the methanol/water (80/20, v/v) mobile phase ($k' \sim 0.2$). The retention was not significantly improved even when the methanol content was increased to 90%. Based on these results, acetonitrile/water (80/20, v/v) was chosen

Table 1
Packing properties of the three amino columns and the chromatographic data for tromethamine

Column	Packing properties			Chromatographic data		
	Carbon load (%)	Surface coverage[a]	End-capping	Capacity factor	Efficiency (plates)	Asymmetry factor
Zorbax NH_2	4.0	3.7	No	2.35	8211	1.52
YMC Pack NH_2	3.4	3.5	No	2.33	5510	0.6
Nucleosil NH_2	3.5	3.1	No	2.42	6146	0.65

[a] In the unit of μmol/m^2.

for the mobile phase. In addition, the high acetonitrile content in the mobile phase also brought extra benefit of increasing the solubility of the investigational drug and its tromethamine salt since samples had to be prepared in the mobile phase for RI detection.

Three amino columns from different vendors were tested with the aim to select the best performing column. Fig. 3 shows the separation of tromethamine and AEPD using the acetonitrile/water (80/20, v/v) mobile phase on three amino columns. The packing properties of the three columns and chromatographic data for tromethamine are presented in Table 1. Zorbax NH_2 column had slightly higher carbon load and surface coverage than YMC-Pack NH_2 and Nucleosil NH_2 columns, but Nucleosil NH_2 column produced a slightly larger retention factor for tromethamine than the other two columns. Zorbax NH_2 column yielded the highest efficiency for tromethamine. Both tromethamine and AEPD peaks showed small tailings on Zorbax NH_2 column, but significant fronting on YMC-Pack NH_2 and Nucleosil NH_2 columns. Therefore, Zorbax NH_2 column was selected for further method development.

3.2. The effect of acetonitrile content

The mobile phase used in HILIC is similar to reverse-phase HPLC, namely, aqueous–organic mixture. However, water is considered as the stronger solvent and an increase in the organic solvent content would lead to longer retention of polar analytes in HILIC separation [6]. In this study, we investigated the effect of acetonitrile content (50–95%) in the mobile phase on retention. No retention was obtained for the analytes under 50% acetonitrile. Fig. 4 shows the plot of ln k' versus acetonitrile content. The retention of both tromethamine and AEPD increased at higher acetonitrile contents, opposite to the behavior in reversed-phase HPLC. The value of ln k' increased in a linear fashion in the range from 60 to 95% acetonitrile for tromethamine and in the range of 65–95% acetonitrile for AEPD. However, the ln k' value for tromethamine at 50% acetonitrile deviated from the linear behavior, and the k' value for AEPD started to deviate from the linear fashion at 60% acetonitrile. Repeated experiments confirmed that the deviation at 50% acetonitrile was not due to the experimental error. This deviation possibly implies that the separation mechanism might not be completely based on hydrophilic interaction, and secondary interactions might be involved at lower acetonitrile content [6].

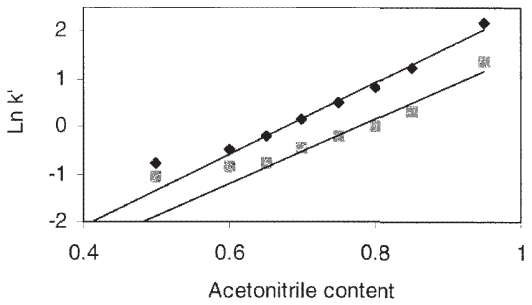

Fig. 4. Plots of ln k' vs. acetonitrile content in the mobile phase for tromethamine (◆) and AEPD (■). Column: Zorbax NH_2, and column temperature: 25 °C.

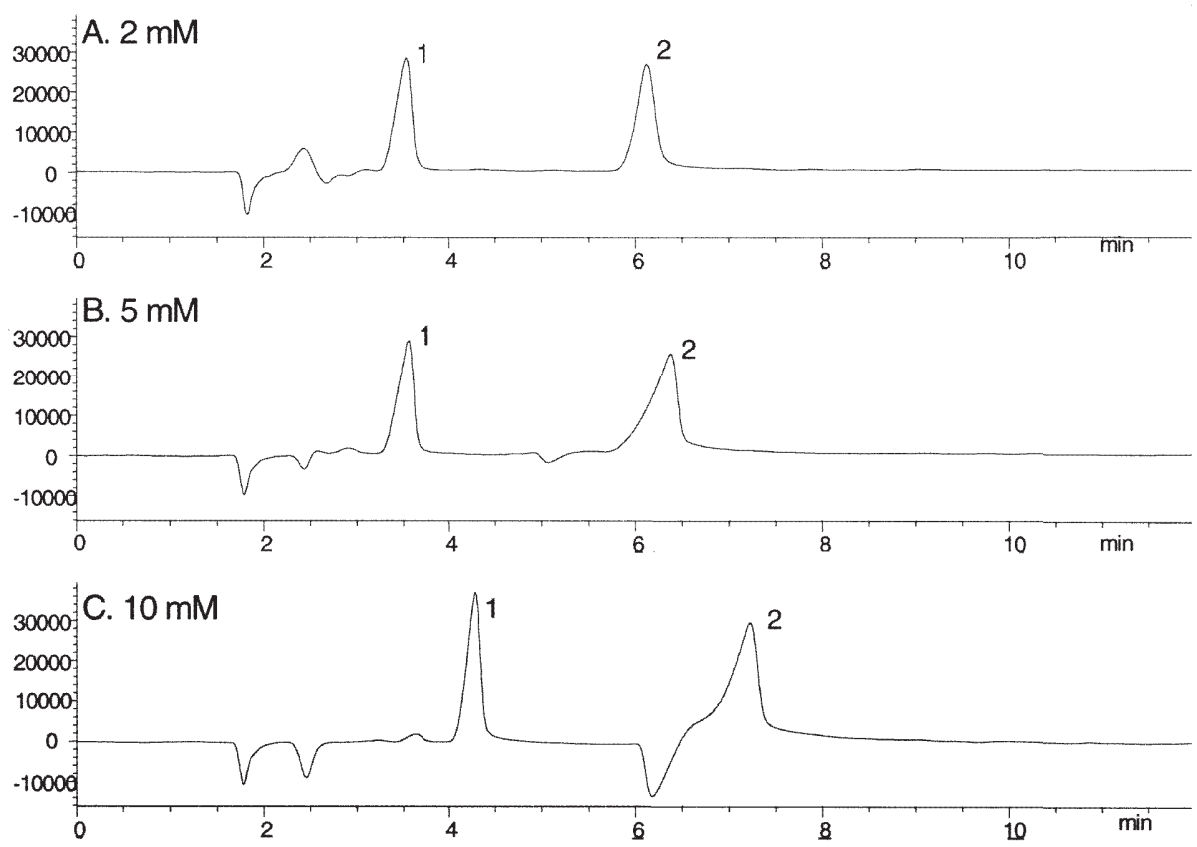

Fig. 5. Chromatograms for the separation of tromethamine and AEPD with different amounts of ammonium acetate in the mobile phase. Mobile phase: acetonitrile/water (80/20, v/v) containing (A) 2 mM, (B) 5 mM, and (C) 10 mM ammonium acetate. Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size. Other conditions are the same as in Fig. 3.

Table 2
Chromatographic data for AEPD and tromethamine on a Zorbax NH_2 column in the mobile phase of acetonitrile/water (80/20, v/v) containing various concentrations of ammonium acetate (NH_4Ac)

NH_4Ac (mM)	AEPD			Tromethamine		
	Capacity factor	Efficiency (plates)	Asymmetry factor	Capacity factor	Efficiency (plates)	Asymmetry factor
2	0.94	1971	0.60	2.36	4654	0.93
5	0.99	1999	0.50	2.56	2463	0.44
10	1.40	3956	0.68	3.06	3162	0.49

3.3. The effect of ammonium acetate

Ammonium acetate is often used as a buffer salt in the mobile phase for HILIC separations due to its good solubility at high organic content [8]. The effect of ammonium acetate on the separation of tromethamine and AEPD was investigated by varying ammonium acetate concentration in the acetonitrile/water mobile phase (80/20, v/v). Zorbax NH_2 column used for this study was first washed with IPA, and then with an acetonitrile/water mixture (50/50, v/v). Finally the column was equilibrated with the mobile phase containing ammonium acetate. The apparent pH of the

mobile phase containing ammonium acetate was not measured, but the pH of the aqueous solution of 10 mM ammonium acetate was about 6.6. Fig. 5 shows the chromatograms for the separation of tromethamine and AEPD using the mobile phase containing 2, 5 and 10 mM ammonium acetate, and the chromatographic data for AEPD and tromethamine are presented in Table 2. The baseline dip before the tromethamine peak in Fig. 5B and 5C was possibly due to contaminants in the ammonium salt since it was also present in the blank injection. It was noticed that the analyte peaks displayed obvious fronting as compared with the separation in the mobile phase without ammonium acetate, and the fronting became more serious at higher ammonium acetate concentration. The possibility of mismatch between the sample solvent and mobile phase causing peak fronting was ruled out since the samples were prepared in the same mobile phase containing ammonium acetate in this study as required by the RI detection. The fronting might be related to the changes in the immobile water layer on the surface of the packing material due to increasing salt concentrations. Furthermore, the capacity factors also increased for both AEPD and tromethamine as the ammonium acetate concentration in the mobile phase was raised from 2 to 10 mM. The acetate counter ions might be adsorbed onto the positively charged amino phase through electrostatic interaction. This could reduce electrostatic repulsion of the positively charged analytes from the positively charged stationary phase, thus resulting in increased retention.

3.4. Effect of column temperature

Column temperature is often evaluated as a useful variable in method development. We investigated the effect of column temperature on retention in the range 15–55 °C. The effect of column temperature on retention was studied by Van't Hoff equation, which describes the dependence of $\ln k'$ on absolute temperature (T):

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi$$

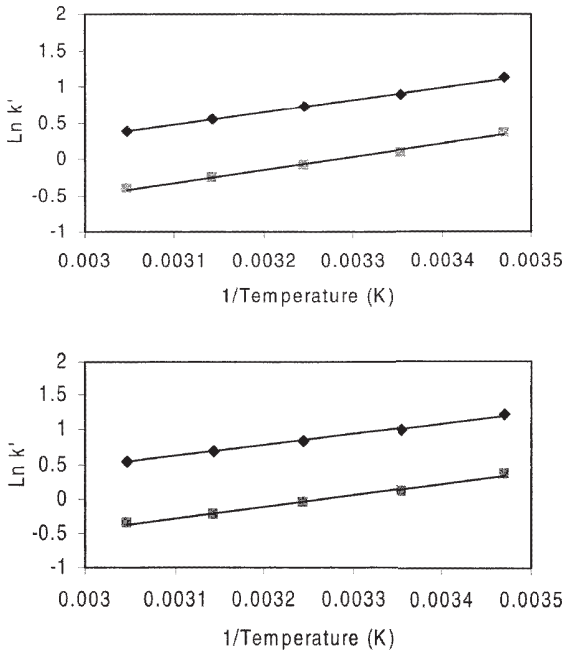

Fig. 6. Plots of $\ln k'$ vs. 1/temperature for tromethamine (◆) and AEPD (■). Top panel: mobile phase without ammonium acetate. Bottom panel: mobile phase with 5 mM ammonium acetate. Mobile phase: acetonitrile/water (80/20, v/v). Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size.

where ΔH° and ΔS° are the retention enthalpy and entropy, R is the gas constant and ϕ is the phase ratio. Fig. 6 shows the Van't Hoff plots for tromethamine and AEPD in the mobile phase of acetonitrile/water (80/20, v/v) with and without 5 mM ammonium acetate. The Van't Hoff plots for both compounds were linear and had a positive slope. The retention for both analytes decreased with increasing column temperature under both mobile phase conditions. The calculated enthalpy values ($-\Delta H^\circ$) were 14.1 ± 0.3 and 15.0 ± 0.7 kJ/mol for tromethamine and AEPD, respectively, on Zorbax NH_2 column in acetonitrile/water (80/20, v/v) mobile phase without ammonium acetate. With 5 mM ammonium acetate in the mobile phase, the enthalpy values changed to 13.4 ± 0.2 and 14.2 ± 0.5 kJ/mol for tromethamine and AEPD, respectively. There was no statistical difference in the enthalpy values for AEPD with or without the presence of 5 mM ammonium acetate in the mobile phase. However, the enthalpy

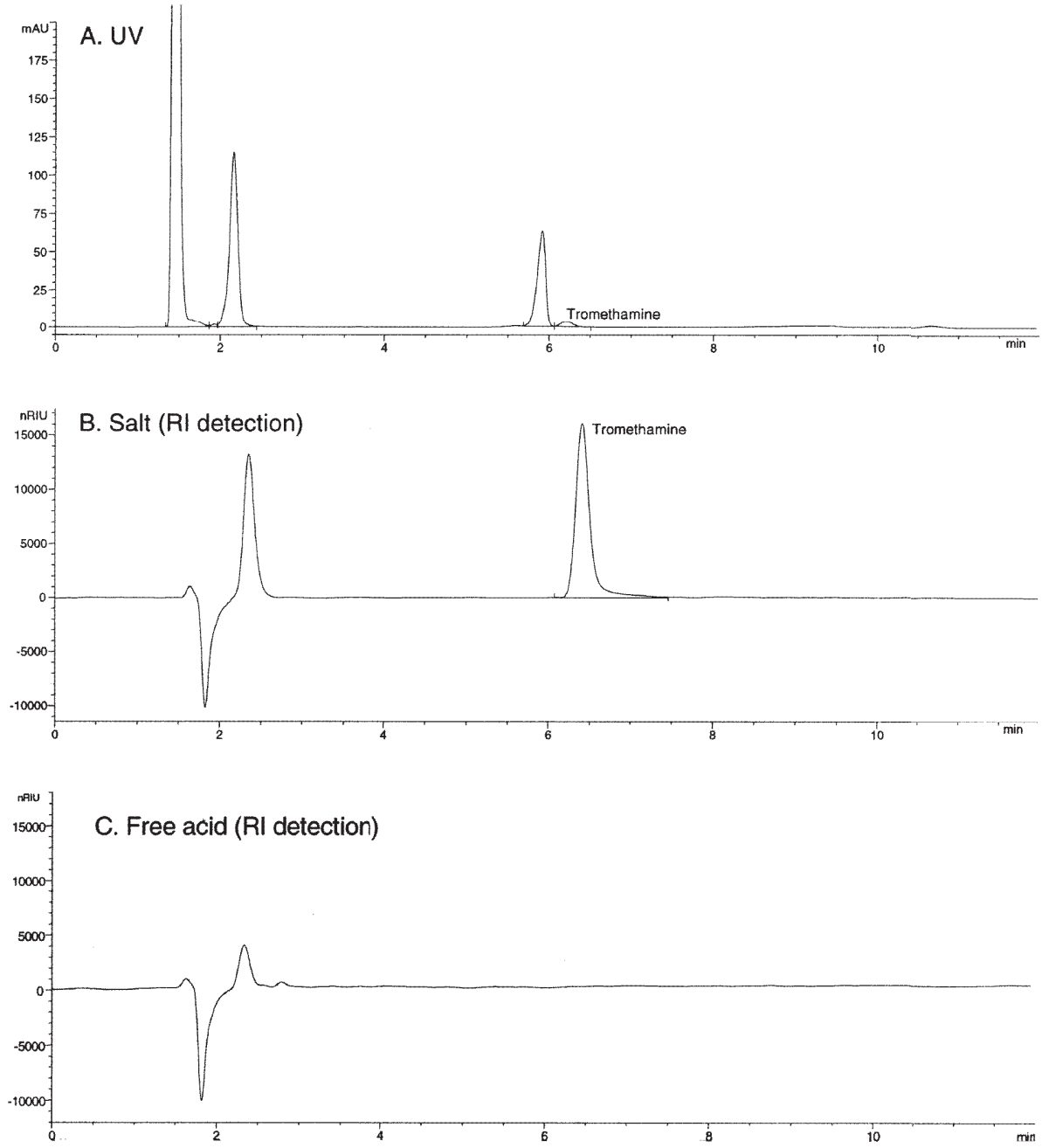

Fig. 7. Chromatograms for the salt and free acid form of the investigational drug from UV and RI detectors. Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size. Column temperature: 25 °C. Mobile phase: acetonitrile/water (80/20, v/v). Flow rate: 1 ml/min. Sample: the investigational API (salt form ~2 mg/ml in the mobile phase). Injection volume: 50 μl.

value for tromethamine in the mobile phase containing 5 mM ammonium acetate was slightly lower (~5%) than that in the mobile phase without ammonium acetate. The enthalpy change might be related to the retention mechanism of the HILIC separation, and may help to explain why

Table 3
Retention time of AEPD and tromethamine (Tris) under varied chromatographic conditions

	Flow rate (ml/min)			Column temperature (°C)			Acetonitrile content (%)		
	0.9	1.0	1.1	22	25	28	78	80	82
AEPD	4.52	4.07	3.72	4.26	4.07	3.96	3.99	4.07	4.58
Tris	7.08	6.37	5.80	6.65	6.37	6.14	5.99	6.37	7.69

the addition of ammonium acetate caused increased retention as discussed above.

3.5. Method validation

3.5.1. Method specificity

The HILIC method was intended for the analysis of tromethamine content in the investigational API. The method specificity was demonstrated by analyzing the free acid form and salt form of the investigational compound with online diode array (DAD) and RI detectors. Fig. 7 shows the chromatograms of the salt form from both UV (225 nm) and RI traces and the chromatogram of the free acid from the RI detector. The large peak eluting in the solvent front (1.4 min) in the UV trace was confirmed by UV spectrum to be the free acid form of the API. Other impurities in the drug substance were also observed in the UV trace, for example, the peaks at 2.2, 5.9 and 10.7 min. The small peak at 6.2 min corresponded to tromethamine. The small difference in the retention times in the UV trace and RI traces (6.2 vs. 6.4 min) was due to the fact that the DAD detector was positioned ahead of the RI detector. The RI trace of the free acid form did not show any peak around the elution time of tromethamine. The comparison of the UV and RI traces demonstrated that the free acid form of the API and related impurities presented no interference to tromethamine detection.

3.5.2. Repeatability, linearity and sensitivity

The method repeatability was validated by using tromethamine reference standard solution. Six injections of a reference standard yielded relative standard deviation (%R.S.D.) of 1.9%, and difference between the average response factors (i.e. standard weight/peak area) of three injections of two reference standards was 0.5%. The linear dynamic range of the RI detector was evaluated using six reference standards ranging from 0.1 to 2.0 mg/ml. Linear regression of peak area versus standard concentration data yielded a correlation coefficient of 0.9997.

Refractive index was chosen as the detection mode for tromethamine to overcome the lack of chromophores. The relative poor sensitivity of the RI detector was partially compensated by a large injection volume (50 μl). A limit of detection of 0.03 mg/ml was obtained based on signal to noise ratio of at least 3. The method sensitivity was not as low as that of UV detection with derivatization or conductivity detection in IC; however, it was sensitive enough for the purpose of this method.

3.5.3. Robustness

The robustness of the HILIC method was investigated by slightly changing the chromatographic conditions such as flow rate, column temperature, and acetonitrile content in the mobile phase. In robustness testing, the flow rate was changed by 10% of the normal value, and the column temperature was varied by 3 °C. The acetonitrile content was shown to have a large effect on the retention time of the analytes (Fig. 3). Therefore, only a small change in the acetonitrile content (2%) was tested. The retention time of AEPD and tromethamine obtained under different robustness test conditions is presented in Table 3. Compared with the retention time under normal running conditions, i.e. flow rate of 1 ml/min and column temperature of 25 °C, the retention time fluctuated by about 10% due to the changes in flow rate and column temperature. When the acetonitrile content was 2% lower than the normal

Table 4
Recovery of tromethamine from the investigational compound

Amount spiked (mg/ml)	Amount recovered (mg/ml)	Average recovery (%)
0.311	0.30	96.5
0.567	0.59	104.1
0.996	0.99	99.4

level (80%), the retention time changed by only 2 and 6% for AEPD and tromethamine, respectively. However, 2% upward shifting of the acetonitrile content caused 13 and 20% change in retention time for AEPD and tromethamine, respectively. This indicated that tromethamine retention time was very sensitive to the variation of the acetonitrile content in the mobile phase, especially to the upward shifting. In addition, more than three Zorbax NH_2 columns were used in this study and variation in retention time and efficiency for tromethamine was less than 10%. For example, the chromatograms in Fig. 2A and Fig. 3A showed the separation of AEPD and tromethamine on two different Zorbax NH_2 columns, and column performance was very comparable.

3.5.4. Recovery

The free acid form of the API was not soluble in the acetonitrile/water mixture (80/20, v/v). Therefore, the recovery study was not performed by spiking the solution of the free acid form with tromethamine at different levels. Instead, tromethamine solutions of different concentrations (0.3, 0.6 and 1.0 mg/ml) were used to dissolve the free acid form of the investigational API at 1.4 mg/ml. The recovery data presented in Table 4 indicated that good recovery was achieved at all the three levels. This demonstrated that the method was accurate to analyze tromethamine in the investigational drug substance.

3.6. Application

The validated method was used to analyze seven small research batches of the investigational drug substance in support of process research on salt formation. Table 5 presents the analytical results for the tromethamine content (w/w%) in the investigational API as well as the potency of the drug substance in the form of free acid (w/w%) using a separate reverse-phase HPLC method. Our results confirmed to synthetic chemists that the salt form of the drug was a bis-tromethamine salt, and at least two equivalents of tromethamine were needed in the salt formation.

4. Conclusions

We have developed a HILIC method for the analysis of tromethamine counter ion in an investigational API. The HILIC approach demonstrated distinct advantages over conventional reverse-phase HPLC for the separation of small polar compounds. In this method, the non-salt form (free acid) of the API eluted in the solvent front. This minimized interference of the active moiety (normally in larger amount) with tro-

Table 5
Tromethamine amount in the salt form of the investigational drug substance

Sample number	Tromethamine (%, w/w)	Free acid (%, w/w)	Molar ratio[a]	Tromethamine added[b]
1	24.9	73.4	1.9	2 Equivalents
2	24.8	76.5	1.9	3 Equivalents
3	27.0	76.2	2.0	4 Equivalents
4	14.8	82.4	1.0	1 Equivalents
5	25.4	77.3	1.8	2 Equivalents
6	25.4	77.2	1.8	2 Equivalents
7	26.2	77.2	1.9	2 Equivalents

[a] The ratio of moles of tromethamine to free acid.
[b] Amount of tromethamine added in the salt formation step of the synthesis.

methamine analysis, but also avoided washing steps that would be necessary if the active moiety were to elute after tromethamine. The mobile phase used in this method not only provided sufficient retention for the analyte, but also solved the solubility problem with the investigational drug. The validation proved that this HILIC method was accurate and precise for the determination of tromethamine at the level expected in the investigational drug substance.

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